Ministry of Higher Education
and Scientific Research
Diyala University
College of Education for Pure Science

Polymerase Chain Reaction For Detection And Genotyping Of *Molluscum Contagiosum* Virus In Diyala Province

A Thesis

Submitted to The Council of The College of Education for Pure Science /Diyala University in Partial Fulfillment of the Requirements for The Degree of Master of Science in Biology /Microbiology

By

Raghad Ibrahim Ahmed

B .Sc. Microbiology

Baghdad University

Supervised by

Assist. Prof .Dr
Mohammed Khalifa AL –Azawi
College of Science
Diyala University

Assist. Prof. Dr.
Khudhair Khalf Ibrahim Al-Kayalli
College of Medicine
Diyala University

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وزارة التعليم العالي والبحث العلمي
جامعة ديالى
كلية التربية للعلوم الصرفة

تفاعل إنزيم البلمره المتسلسل لكشف التنميط الجيني لفيروس المليساء المعدية في محافظة ديالى

رسالة تقدمت بها
مجلس كلية التربية للعلوم الصرفة /جامعة ديالى
وهي جزء من متطلبات شهادة الماجستير قي علوم الحياة /
الإحياء المجهرية

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بكالوريو علوم حياة / حيا مجهريه

. . . محمد خليفة
كلية العلوم
جامـــــــعة ديالى

. . .خضير خلف ابراهيم الكيالي
كلية الطب
جامـــــــعة ديالى

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صدق الله العظيم

سورة النور / الآية ٣٥

Supervisors' Certification

We, certify that this thesis entitled **Polymerase Chain Reaction For Detection And Genotyping Of Molluscum Contagiosum Virus In Diyala Province** that presented by the student **Raghad Ibrahim Ahmad** was prepared under our supervision at the Department Of Biology-College of Education for Pure Science–Diyala University as a partial fulfillment of the requirements for the degree of Master of Science in Biology /Microbiology .

Supervisor	**Co- Supervisor**
Assist Prof. Dr.	**Assist Prof.Dr.**
Mohammed Khalifa AL-Azawi	**Khudhair Khalf Ibrahim Al-Kayalli**
Date : / /2013	**Date : / /2013**
College of Science	**College of Medicine**
Diyala University	**Diyala University**

-In view of the available recommendation, I forward this thesis for debate by the examination committee.

Signature :
Assistant Prof.
Name : Dr. Najim Abdullah Al-Zubaidy
Date : / /2013
Head of the department of Biology /College of Education for Pure Science .
Diyala University

Linguistic Certificate

I certify this thesis entitled **Polymerase Chain Reaction For Detection And Genotyping Of Molluscum Contagiosum Virus In Diyala Province**," was presented by "**Raghad Ibrahim Ahmed**" and corrected linguistically by me

Signature

Name :

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Scientific Certificate

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We, Examining Committee, certify that we have read this thesis entitled **Polymerase Chain Reaction For Detection And Genotyping Of Molluscum Contagiosum Virus In Diyala Province** and have examined the student **Raghad Ibrahim Ahmad** in its contents . We thank it is adequate as a thesis with standing (Excellent) for the degree of Master of Science in Biology /Microbiology.

Signature
Professor Dr.
Name :Abas Aboud Farhan
Date : / / 2013
Chairman

Signature
Assist Prof .Dr.
Name. Saad Hasan Mohammed Ali
Date : / / 2013:
Member

Signature Dr.
Assist Prof .Dr.
Name: Nadhim Ghazal Noaman Alammar
Date : / / 2013
Member

Signature
Assist Prof. Dr.
Name : Mohammed Khalifa AL-Azawi
Date : / / 2013
Memberand Supervisor

Signature
Assist Prof .Dr.
Name : Khudhair Khalf Ibrahim Al-Kayalli
Date: / / 2013:
Member and Co-Supervisor

Signature
Dean of College
Professor .Dr.
Name :Abas Aboud Farhan
Date : / / 2013

Dedication

To . . .

The light of my way . . . . Mother and Father . . .

To . . . Who supported me to complete my studies on . . . . My husband

To . . . the bright stars in my sky

My dear . . . . My single brother Ala'a . . . and my sisters Maha , Rana and Noor . . . .

To . . . .

My Heart . . . . Rawan , Ali and Humam . . . . .

To . . .

My uncles and aunt . . . . . .

To everyone who aided me in every possible way to make this work see the light.

Raghad . . .

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Thanks go to the Staff of the Public Health Laboratories, teaching Baqubah Hospital and my friends Dr. fatima ,shimaa and suhad ,

خلاصـــــــة البحث

أجريت المقطعية الحالية /تشرين / ولغاية

/نيسان / العيادة الاستشارية التعليمي. هدفت

بين التشخيص ألسريري بالفيروس التشخيص

ية (Polymerase Chain Reaction)

تحديد ديالى .

تشخيص ستين( ) مريض بفيروس المليساء المعدية

(-) (. %)

مريض (. %) .

(%) عينات

نتيجة لفيروس المليساء المعدية (. %) عينات

نتيجة الفيروس .

بينت (. %) العمرية (-

) (. %) (. %) ولا يوج

بين بالفيروس .

بينت للفيروس هو " (. %)

(. %) الفيروس

(. %) العمرية (-) ليصل

(. %)،بينما للفيروس (%) بين

العمرية . ين

الفيروس .

أظهرت (. %) بالفيروس بين المرضى غير

المتعلمين ،وتبين انه لايوجد فرق بين بالفيروس

ومستوى التعليم .

بينت الدراسة (. %) بالفيروس موقعها

(. %) في الجانب الايمن من الـ (. %)

المصابين اقل اويساوي مواقع للاصابة ،لايوجد فرق معنوي بين الاصابة

بالفيروس وموقعها وعدد مواقع الاصابة .كذلك بينت الدراسة بين

المرضى في المدينة المرضى في الريف ،

.

Appendices

Name:

Age :

sex :

Address :

Province : district: sub district: village :

Educational level

Illiterate: Primary School : Secondary School:

College :

Site of lesions

Head and neck: Genital area : Other body site :

Side of lesions

Right : Lift :

Number of lesions

Chapter one

Introduction

Chapter two

Literature review

Chapter three

Materials and Methods

Chapter four

Results and Discussion

Conclusions and Recommendations

References

1. Introduction and Aims of study

1.1. Introduction:-

Molluscum contagiosum virus (MCV): is a viral skin infection which may infect the mucous membrane, occasionally, It is Molluscipox virus from family Poxviridae. Molluscum contagiosumvirus (MCV) was first described and later assigned its name by Bateman in the beginning of the nineteenth century (Bateman *et al*.,1953.)

In 1841 Henderson and Paterson described the intracytoplasmic inclusion bodies now known as molluscum or Henderson-Paterson bodies.In the early twentieth century, Juliusberg, Wile, and Kingery were able to extract filterable virus from lesions and show transmissibility (Juliusberg *etal.,*1905).

MCV has no animal reservoir, infecting only humans and there are four types of MCV, MCV-1,MCV-2,MCV-3 andMCV-4. MCV -1 was the most prevalent predominantly seen in children and MCV -2 was seen usually in adults and often sexually transmitted (Hanson *et al.*, 2003).

Sites of predilection were face, eyelids, neck, axillae thighs and genital area in sexual contact. In Acquired Immunodeficiency Syndrome (AIDs) the lesions are large, numerous and on the face. Mild erythema or eczematous dermatitis may develop around the papules. Primary mode of transmission was via direct human contact including sexual means lead to the appearance of lesions in the genital areas, contaminated fomites, and autoinoculation through scratching is also suspected (Stulberg*et a*l; 2003).

The diagnosis of MCV was usually done clinically. They need for laboratory diagnosis of MCV was speculative, because a spontaneous healing was observed in cases where no underlying immune defect is present, the disease was considered as a self-limiting condition. However, there were several lines of reasoning where medical intervention and treatment might be beneficial. Though molluscum cannot be cultured in the laboratory.

Histological examination of a curetted or biopsied lesion can also used in the diagnosis in cases that are not clinically clear. The thick white central core can be expressed and smeared on a slide and left unstained or stained with Geimsa or Gram stains to demononstrate the large brick-shaped inclusion bodies. Electron microscopy has also been used to demonstrate MCV structures. Immunohistochemical methods' using a polyclonal antibody allows recognition of molluscum contagiosum in fixed tissue. In-situ hybridization for MCV DNA has also been utilized(Bikowski;2004).

The best option for the definitive diagnosis of MCV was PCR-based assays. An additional benefit of molecular diagnosis was the results provide information about the type of the infecting molluscum strain. No molecular data have been reported in the literature regarding prevalence of MCV types in Iraq . Thus, in this study we attempted to document the feasibility of DNA amplification-based assay in the clinical laboratory.

1.2. Aims of study:-

1. To confirm the clinical diagnosis of MCV by laboratory test through using PCR assay.
2. To know the predominate type of MCV that found in Diyala Province.

3. Patients, Materials and Methods

3.1 Materials:-

3.1.1. Laboratory equipments and instruments:-

Table(3.1) List of Laboratory equipments and instruments:

No	Apparatus	Manufactured company
1.	Autoclave	Biosan(USA)
2.	Cold rack box	Biobasic (USA)
3.	Deepfreeze -37C	Hemar (France)
4.	Digital camera	Sony(Japan)
5.	Eppendrof rack tube	Bioneer(Korea)
6.	Eppendrof centrifuge	Hettich (Germany)
7.	Eppindorf tube	Promega(USA)
8.	Gel electrophoresis apparatus	Optima (Japan)
9.	Graduated cylinders.	Jlasssco (India)
10.	Graduated pipettes0.1-2micro liter	Chance Proper Ltd(K.U)
11.	Graduated pipettes0.5-10 micro liter	Chance Proper Ltd(K.U)
12.	Graduated pipettes200-1000 micro liter	Chance Proper Ltd(K.U)
13.	Graduated pipettes20-200 micro liter	Chance Proper Ltd(K.U)
14.	Graduated pipettes2-20 micro liter	Chance Proper Ltd(K.U)
15.	Hood	Shinsaeny(Korea)
16.	Hot plate	Memmert ,Co. Ltd.(Germany)
17.	Maneral curate	Blinder /USA
18.	Oven	Blinder (USA)
19.	PCR system(Thermo cycler)	Eppendrorf (AG) Germany
20.	PH meter	Metller; Switzerland
21.	Pipette tips blue	Bioneer (Korea)

22.	Pipette tips yellow	Bioneer(korea)
23.	Pipitte tips blue	Bioneer (Korea)
24.	Refrigerator	(Turkish)
25.	Sensitive balance	Sartorius (Germany)
26.	Shaker water bath	Memmert, Co. Ltd.(Germany)
27.	Volumetric flask	Jlasssco (India)
28.	Vortex mixter	Heldoph(Germany)
29.	Water stillerization (distellater)	Jlabatech (Korea)

3.1.2.Chemicals:

3.1.2.1Laboratory chemical Materials :-

Table(3.2) List Of Laboratory Chemical Materials :-

No	Chemicals	Manufactured company
1.	Absolute Ethanol	BDH(England)
2.	Absolute ethanol solution (100%)	BDHChemicalLtd (England)
3.	Accu Power PCR Pre Mix	Bioneer(Korea)
4.	Agarose	Bioneer (Korea)
5.	Bam.H1	Promega (USA)
6.	Deionized distilled water	Bioneer (Korea)
7.	Di-Sodium Hydrogen Orthophosphate (Na2HPO4)	Analar(England)
8.	Distilled water.	Bioneer (UAS)
9.	DNA ladder	Bioneer (Korea)
10.	DNA purification kit	Geneaid (Ttwain)

No	Chemicals	Manufactured company
11.	Ethidium bromide	BDH-Liverpool(England)
12.	Go Taq Green Master Mix PCR	Promega(USA)
13.	Hydrochloric acid(HCL)	BDH(England)
14.	Loading dye	Biobasic (USA)
15.	Magnesium chloride ($Mg\ Cl_2$)	Analar(England)
16.	Potassium chloride(KCl)	Merck(Denmark)
17.	Potassium Di-Hydrogen Phosphate (KH2PO 4)	Riedal-De Haenag Seelze –Hannover(Germany)
18.	Primer	Bioneer(Korea)
19.	Sodium chloride(NaCl)	Fisher cientific International Company (UK).
20.	TBE10X	Biobasic(USA)

3.1.3.Laboratory diagnostic kits :

Table (3.3) List of Laboratory diagnostic DNA extraction kit (Genomic DNAMini Kit(Tissue))

NO.	Name	Manufactured company
1	2ml collection Tube	**Gonaid** (Genomic DNA Mini Kit (Tissue)
2	GBT Buffer	**Gonaid** (Genomic DNA Mini Kit (Tissue)
3	GD Column	**Gonaid** (Genomic DNA Mini Kit (Tissue)
4	GT Buffer	**Gonaid** (Genomic DNA Mini Kit (Tissue)
5	Micro pestle	**Gonaid** (Genomic DNA Mini Kit (Tissue)
6	Proteinase K	**Gonaid** (Genomic DNA Mini Kit (Tissue)
7	W1 Buffer	**Gonaid** (Genomic DNA Mini Kit (Tissue)

3.2 Methods:

3.2.1 Preparation of solution buffer that used to collect samples :-

3.2.1.a.Phosphate buffer saline(PBS):-

This solution was prepared according to the method described by (Dulebecco et al.,1954) . Phosphate Buffered Saline is the simplest of basic salt solution and was used for collection of the samples. PBS Solution was prepared from the following ingredients: For 1 liter of 1X PBS, prepare as follows:

1. Start with 800 ml of distilled water:
2. Add 8 g of NaCl.
3. Add 0.2 g of KCl.
4. Add 1.44 g of Na_2HPO_4.
5. Add 0.24 g of KH_2PO_4.
6. Adjust the pH to 7.1 with HCl.
7. Add distilled water to a total volume of 1 liter.

The pH was adjusted to 7.2. Dispense the solution into aliquots and sterilize them by autoclaving (20 min, 121°C, 63pond/cm^3) for 30 mint and kept at 4℃.

3.3.Collection of samples:-

This study was conducted in outpatient Clinic of Dermatology of Baquba Teaching Hospital as across section study including all patients atteuding in the period between 1 November 2011 to 3o of April 2012. the collection of patients sample was done in dermatology unit by specialist dermatologist who diagnosis the cases . the sample was take and diagnosis by PCR.

The demographic information include age, sex, address, educational status, and last the number and the distribution of lesions present was recorded.

The lesion from each patient was curetted and placed in 5 ml phosphate buffered saline, pH 7.1, and immediately transported to the laboratory. The samples were stored at -37˚C until theextraction the DNA.

Conventional Polymerase chain reaction was used to detection the Molluscum contagiosum virus, and resection enzyme Bam.HI to type of virus . Sixty (60) samples were selected depending on the size of lesions of the patient, the size of lesion not less than 30mg depended on method as described by geneaid compony

3.4. Characterization of DNA :-

It was followed the way as described by the Sambrook (1989) to measure the concentration and estimate the purity of DNA, as follows: -

DNA extract was reduced to 100 times using distilled water and add 20Microliters of DNA to 1980 Microleter of distilled water, the sample was measured by absorbency diluted sample ultraviolet(UV) Spectrophotometer device measuring optical density and wavelength at 260 nm and 280 nm, then the concentration of DNA was estimated in the sample by applying the following law:

Concentration Of DNA μ g / ml = optical density$_{(260)}$ × inverted of dilution(100) × 50 mg / ml

On the basis that the reading at 260 nm which is equal to 1 equivalent concentration of double-strand DNA of DNA quantity 50 mg / ml.

3.5.Measure the purity of DNA: -

DNA was measured by the degree of purity of the DNA of dividing the optical density at 260 nm wavelength on the optical density measured at 280 nm wavelength where the best purity of DNA between (1.8 -2).

3.6. DNA Extraction:-

Sixty samples were selected depending on their size and lack of contamination of the blood of the patient. and after neglecting the PBS, the DNA extraction and purification as instructed by the Geneaid company.

3.6.1. Preparing solution used in Genomic DNA Isolation:-

Before starting by the extraction we will prepared two solutions to use it in our process.

3.6.1.a. Protienase K:-

It was Prepared by adding deionize distill water ($ddH_2 0$) 1.5 milliliter (ml) to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4º C protienase K used to lyses the protein .

3.6.1.b..Wash Buffer:-

Was prepared by adding absolute ethanol (100 ml) to wash buffer and store at room temperature used to remove any contamination **.**

3.6.1.c. Step Of Extraction

- **Step1:- . Tissue Dissociation:-**

1-Thirty (30) milligram of human skin tissue was cutoff and transfer it to a 1.5 ml micro -centrifuge tube.

2- Use the provided Micro pestle to grind the tissue to a pulp.

3- Add 200 µl of GT Buffer to the tube and continue to homogenize the sample tissue by grinding.

- **Step 2:- Lysis:-**

1- Twenty µl (microleter) of Proteinase K was added to the sample mixture and mix by shaking vigorously. and incubate at 60°C for 30 minutes to lys the sample. During incubation, invert the tube every 5 minutes.

2- Two hundred (200) µl of GBT Buffer was added and mix by shaking vigorously for 5 seconds. Incubate at 70°C for at least 20 minutes to ensure that the sample lysate was clear.

During incubation, the tube was inverted every 5 minutes. At this time, pre-heat the required Elution Buffer (200 µl per sample) to 60 °C for Step 5 DNA Elution.

If there is insoluble material present following incubation, centrifuge for 2 minutes at 14-16,000 x g and transfer the supernatant to a new 1.5 ml micro centrifuge tube.

- **Step 3:- DNA Binding**

1- Two hundred µl of absolute ethanol was added to the sample lyses and immediately mixed by shaking vigorously for 10 seconds.(If precipitate appears, break it up by pipe ting).

2- Place a GD Column in a 2 ml Collection Tube.

3- All of the mixture was transfer (including any precipitate) to the GD Column.

4- At 14-16,000 x g for 2 minutes was centrifuging.

5- The 2 ml collection tube was discarded containing the flow-through and transfer the GD Column to a new 2 ml collection tube**.**

- **Step 4:- Washing**

1-Add 400 µl of W1 Buffer to the GD Column.

2- Centrifuge at 14-16,000 x g for 30 seconds and discard the flow-through.

3- Place the GD Column back in the 2 ml Collection Tube.

4- Add 600 µl of Wash Buffer (ethanol added) to the GD Column.

5- Centrifuge at 14,000-16,000 xg for 30 seconds and discard the flow-through.
6- Place the GD Column back in the 2 ml Collection Tube
7-Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix.

- **Step5:-DNA Elution:-**

The Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.
1-The dried GD Column transferred to a clean 1.5 ml micro centrifuge tube.
2- Add 100 µl of pre-heated Elution Buffer or TE to the center of the column matrix.
3- Let stand for at least 5 minutes to ensure the Elution Buffer or TE is absorbed by the matrix.
4- Centrifuged at 14-16,000 x g for 30 seconds to elute the purified DNA

3.7.Primer selection:

Table (3.4) showes two sets of primers that were used in the study as suggested by (Nunez et al ;1996)

Table (3.4)primers used in the study.

Set No.	Primer	No .of bp.	Company
1	F1(5-GGCGCGTAGCCGAGCGG-3) R1(5 CTTCCGGGCTTGCCGCCGGGCAG-3)	393-bp	Bioneer
2	KU (5-GGAGGAGTGCCCATCAAGAAT-3) OR (5-GCTTTTCAGTTTTTGTGCGA-3)	575-bp	Bioneer

NO. bp: Number of base pair

The first primers F1 and R1 amplify 393- base pair (bp) portion of p43K polypeptide from MCV genome whereas KUF and OR primers

amplify 575-bp-long region from p43K polypeptide of MCV genome. KU and OR primers amplified region containing BamH1 restriction site in sub-type genome of MCV, thus allowing accurate sub typing of the infecting strain.

3.8.Polymerase Chain Reaction (PCR)

Conventional PCR was the method that applicated on our samples to obtain result. Doubled polymerization process have been performed twice time , first PCR to use a primer of first set (F1 and R1) which was used for the diagnosis of molluscum contagiosum and saw any lesions positive or negative, the second set another detection to MCV and find out what kind of molluscum contagiosum was prevalent by using restricted enzyme Bam.H1 after amplified region allowing to digestion. Doubled amliphcation had already been done by (Yuns et al ;2006) with simple modification .

3.8.A-The first thermo cycle PCR process include :-

1-Two micro liters of isolated DNA was added to 0.2 of a PCR Pre mix. .PCR Pre mix.kit was selected from bioneer (bioneer/ korea).

2- This mixture (Table 3.5.) containing 10 µM Tris-HCl (pH 8.3), 30 µM KCl, 1.5 mM $MgCl_2$, each deoxynucleoside triphosphate at a concentration of 250 µM, 1U of Taq polymerase, 30 pmol (picomol) of primers F1 and R1, and the mixture was complete to 20 micoleter Deionize distill water (D.D water) .

3-The samples were used with a thermal phases involving initial denaturation at 95°C for 1 min and 40 cycles consisting of denaturation at 95°C for 1mint , annealing at 58°C for 1 min, and extension at 72°C for 1 min, after complete thermo cycle ,extension at 72°C for 5 mint and finally hold the reaction at 4°Cfor 5 mint . these steps were demonstrated in (Table 3.6.),(Fig 3.6.).

4- The amplification reactions were visualized on a 1.5 % agarose.

Table (3.5) First PCR mixture

PCR MIX	Volume
Pre master mix	0.2µL
DNA	2 µL
Primer forword (F1)	3 µL
Primer reverse (R1)	3 µL
D.D.water	12 µL
Total volume	20 µL

Table (3.6) Steps of first PCR program:

PCR program	Time	° C	No. of cycle
Initial denaturation	1 min	95 ° C	
Denaturation	1 min	95 ° C	40 cycles
Annealing	1 min	58 ° C	
Extension	1 min	72 ° C	
Extension	5mint	72 ° C	
Hold	4mint	4° C	

3.8.A.1.<u>Initial Denaturation:-</u>

Prior to the first cycle, the mixture was heated at 95°C for 1 min. to ensure that the DNA strands as well as the primers have melted, so both the template DNA and the primers have completely separated and become single stranded.

3.8.A.2. PCR Steps:

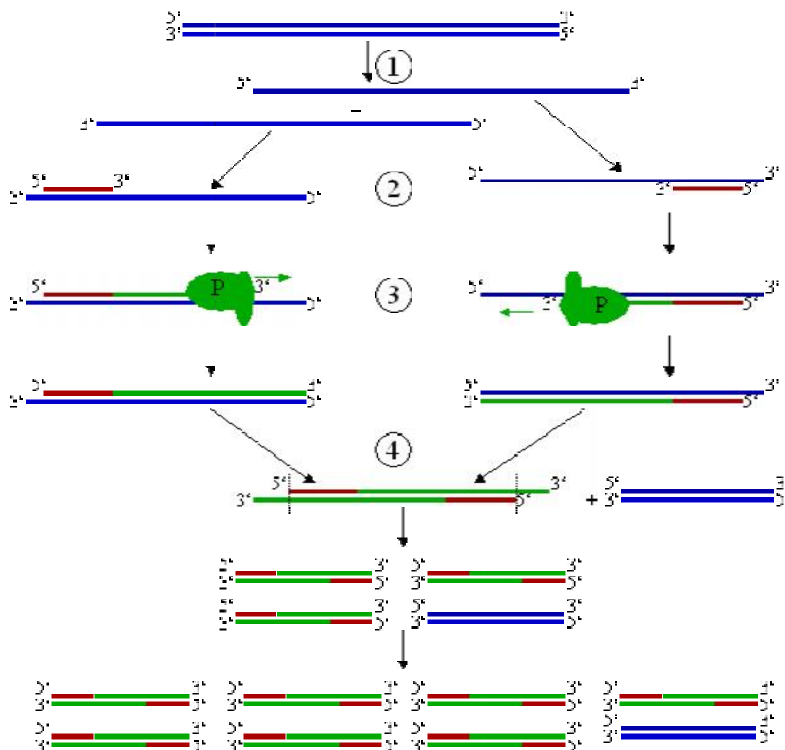
Our PCR process consists of a series of forty cycles. Each cycle consists of three steps (Fig. 3.1).

3.8.A.2.1. Denaturation:

The DNA sample was heated to 95 °C for 1 min. for each cycle in order to disconnect the strands; it breaks apart of the hydrogen bonds that connect the two DNA strands.

3.8.A. 2.2. Annealing:

After separating the DNA strands, the temperature was lowered, so the primers can attach themselves to the single DNA strands. The temperature of this stage depends on the primers and is usually 72°C below their melting temperature, so the temperature used was 58 °C for 1 min. A wrong temperature during the annealing step can result in primers not binding to the template DNA at all, or binding at systematic, the primers are jiggling around, and short bonds are constantly formed between the single stranded primer and the single stranded template.



Fig(3.1) Steps of Polymerase Chain Reaction

3.8.A. 2.3. Extension:

Finally the sample heated at 72 °C for 1 min. The DNA polymerase to starts copy the DNA strands. It starts at the annealed primer and works its way along the DNA strand. The Taq polymerase elongates optimally at a temperature of 72 °C, after complete thermocycle ,extention at 72°C for 5 mintand and the time for this step depends both on the DNA polymerase itself and on the length of the DNA fragment to be amplified .The PCR products were identified by their size using agarose gel electrophoresis. The size of the PCR products were determined by comparing them with a DNA ladder (100bp DNA Ladder Lambda) which contains DNA fragments of known size.

3.8.A.3. PCR Optimization:-

Since PCR is very sensitive, adequate measures to avoid contamination from other DNA may present in laboratory environment (bacteria, viruses, own DNA etc.) were taken. The DNA samples preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction product analysis, were performed in separate areas. For the preparation of reaction mixture, a laminar flow cabinet with UV lamp was used; fresh gloves used for each PCR step as well as used micropipettes with sterile tips. The reagents for PCR were prepared separately in ice and used solely for this purpose and also used optimum concentrations.

3.8.A.4. Electrophoresis (Detection):

3.8.A.4. 1.Solutions and buffers used in electrophoresis:-

3.8.A.4.1.1.Running Buffer (TBE-buffer):-

We have stoke of (Tris -biorate –EDTA)10X .Each 1X contain t of :-- Tries base(0.089M), Boric acid (0.089M) and EDTA(0.0002M). To perpare 1X of teb we take 100 of TEB and add 900ml of distell water to optene finallconstration 1x per later . The pH was adjusted to 8.3 with (HCL)1M.

3.8.A.4.1.2.Stock of Ethedium Bromide :-

This solution was prepared according to the method described by (Maniatis et al;1989).The melting of 1.5 mg of powder dye ethidiom bromide powder in TEB-buffer mixed kept in a bottle and save

at the dark on a temperature 4°C until use and then use the addition of 40 mg / liter of sterile water.

3.8.A.4.1.3. Laoding Buffer (Loading –EZ Dyes And Buffer):-

It was stoke consist of (0.25% Bromophenol blue ;0.25% Xylene Cyanol;60%Glyserol) and its kept at 4° C.

3.8.A.4.1.4.Ladder(100bp DNA Ladder lambda):-

Storage Buffer consist of (10 µM Tris –HCl (Ph8.0),1µM EDTA,2.5% Ficoll,0.005%Bromphenol Blue ,0.005% Xyline Cyanol which is ready to used by adding 10 µl in will .

3.8.A.5. Preparation of Agarose gel:-

It was prepared according to (Phuchareon et al., 1999).

1- Agarose gel 1.5% was prepared by dissolving 2.25g agarose in 150 ml of 1x TBE-buffer by boiling them on hotplate sterrier 100 ºC. until completely melted.

2- The agarose mixture was cooled to 60-65ºC at room temperature; this was followed by addition 3µl of ethidium bromide, the concentration of ethidium bromide stock solution was 10 mg/ml. .

3-The electrophoretic tray was prepared by sealing the gel chamber ends with sticky plastic tapes.

4- Then the mixture was poured gently onto tray and left to solidify at room temperature for 1 hour with inserting the comb at one side of the gel, about 5-10 mm from the border of the gel.

5- When the gel had cooled down and became solid, carefully the comb was removed by gently pulling it straight up with the tray surrounding tapes (end-blocks).

3.8.A.6. Loading DNA samples:-

1-In a sterile eppendorf tube or on a piece of tape (the surface of desk was sterilized), 10 µL of the DNA sample by using a micropipette and transferred onto the gel slots. All the samples were loaded onto the gel in addition to DNA positive control, (Fig 3.5).

2-10 µL of ladder, 100bp DNA Ladder (100-2000bp) was used as a marker, by using a micropipette and transferred onto the gel slot.

3-The gel, with the tray was laid into the chamber with 1x TBE. Make sure the gel was completely covered with TBE, until top surface of gel is submerged approximately 2 mm. and that the slots are at the electrode that will have the negative current.

4-Carefully the safety cover was placed onto the chamber, ensuring that both plugs are secure, and connected with the power supply.
5-Electrophoresis condition was set up at 60 volts for 90 minute,
6-Turn off the power supply, and disconnect the leads.
7-The gel for DNA bands was observed by examining the gel under UV light with protective glasses. And take picture to gel by digital camera.

3.8.B.Asecond PCR amplification and Bam.HI digestion of amplified products:-

1-Three microliters of isolated DNA was added to 25µl of a Green Master Mix , PCR reaction kit was selected from the promega (promega /USA).
2-This mixture (Table 3.5.) containing 10 mM Tris-HCl (pH 8.5), , 3 mM $MgCl_2$, each deoxynucleoside triphosphate(dATP, dGTP, dCTP, dTTP) at a concentration of 400 µM, 1.25 U of Taq polymerase, 30picomol(pmol) of primers KuF and OR1, and the mixter was complete to 50 micoleter Deionized distilled water (D.D water) .
3-The samples was used with a thermal phases involving initial denaturation at 95°C for 1 min and 40 cycles consisting of denaturation at 95°C for 1mint , annealing at 58°C for 1 min, and extension at 72°C for 1 min, after complete thermocycle ,extention at 72°C for 5 mintand finally hold the reaction at 4°Cfor 5 mint . These steps were demonstrated in (Table 3.6.),(Fig 3.7.).
4- The amplification reactions were visualized on a(2%) agarose gel.

Table 3.5 . Second PCR mixture

PCR MIX	Volume
Pre master mix 2X	25µL
DNA	3 µL
Primer forword (F1)	3 µL
Primer reverse (R1)	3 µL
D.D.water	16 µL
Total volume	50 µL

Table 3.6. Steps of second PCR program:

PCR program	Time	° C	No. of cycle
Initial denaturation	1 min	95 ° C	
Denaturation	1 min	95 ° C	**40 cycles**
Annealing	1 min	58 ° C	
Extension	1 min	72 ° C	
Extension	5mint	72 ° C	
Hold	4mint	4° C	

4- The amplification reactions were visualized on a 1.2% agarose gel.

3.8.B.1Ethanol Precipitation Of DNA :

Ethanol precipitation Of DNA carried out according to the method of Sambrook *et al* (1989).the salt concentration of the viral DNA samples was balance by addition of ($MgCl_2$) to final concentration of 0.01M and ($NaCl_2$) to final concentration 0.2M . The DNA sample put in to small volume (300) in eppendrof tube. Three volumes of cold (-20°C) absolute ethanol were added to one volume of salt –adjusted DNA sample . The content mixed gently by using micropipette. The DNA ethanol mixture was then kept at (-20°C) overnight and the precipitation DNA was pelleted by centrifugation at 10,000 RPM(rondom per cycle) for 30 minute in eppendrof centrifuge at 4 ° C . The supernatant was gently aspirated and the pellet was re suspended in cold ethanol 70% ethanol . The DNA suspension was centrifuged as above and the pellet was drain in room temperature before the pellet was resuspended in TE buffer(PH 7.8)

3.8.B.2.Enzyme and Buffer :-

One restriction endonuclease enzyme were obtained from promega company /USA .That enzyme was Bam.H1 .

Bam. H1. consist of (Restriction Enzyme 10X Buffer, Acetylated BSA, 10µg/µl.

33.8.B.3.Digestion :-

About 40µl of purified DNA were ethanol precipitation , pelleted and draind as described previously .The DNA digestion with Bam.H1 Restriction Enzyme, 10u/µl).

1. In a sterile tube, assemble the following components in the order listed below.

Table (3.7) Method of digestion by Bam.H1

Component	Volume
Sterile, de ionized water	16.3µl
Restriction Enzyme 10X Buffer	2µl
Acetylated BSA, 10µg/µl	0.2µl
DNA, 1µg/µl	1.0µl

Mix by pipe ting, then add:-

Restriction Enzyme, 10u/µl	0.5µl
Final volume	20µl

2.Mix gently by pipeting, close the tube and centrifuge for a few seconds in a micro centrifuge. Incubate at the enzyme's optimum temperature for 1.5-2 hours.

3.Add loading buffer to a 1X final concentration and proceed to gel analysis(1.2% final concentration of agarose gel).in volt 60and 90 mint .

Note:-We don't needed Overall night digestions are usually unnecessary and may result in DNA degradation. As protocol of promega .

3.9.Statistical analysis:-

Data analysis was computer aided .statistical analysis were done using SPSS (Statistical Package of social Science) version 18 computer software .Frequency distribution and percentage for selected variable were done. The Chi-square test was used and .X^2. Value (less than 0.05). (Nisi ;2004)

4. Results and Discussion

4. 1 Results:

4.1.1.Study groups:

Ninety two (92) patients were infected with Molluscum contagiosum in different areas of the body were seen in outpatient clinic of Baquba Teching Hospital for the period 1/November /2011 to 30/April /2012,

Sixty (60) patients were selected depending on the size of the lesion was their ages ranged from (1- 80) years , (14; 23.4%) patients in age group (10years), (3; 5%) in age group (11-20years) , (9;15%) in age group(21-30years), (27; 45%) in age group (31- 40years), (5; 8.3%) in age group (41-50years) and (2; 3.3%) in age group (51yeras).

Forty(66.7%) patients male and (20;33.3%) females, , (41; 68.3%) patients from urban area and(19;31.7) from the rural area .

Regarding educational status of the patient (22; 36.7)were illiterate , (21;35%) with primary school level, (9; 15%) with secondary school level and (8; 13.3%) of college level .

Most of the lesions were located on the head and neck of patients (48; 80%), (5;8.3%) on genital area and (7;11.7%) on other site of body , (45; 75%) of lesions on the right side of body and (15;25%) on the left side .

Forty five (75%) of patients had (10)lesions, (6;10%)had (11-20) lesions , (1; 1.7) had (21-30) lesions, (1; 1.7) had (31-40)lesions , (4; 6.7%) had (41-50)lesions and (3;5%) had(51) lesions , (Table l4.1)

Table (4.1) Distribution of variables of patient (n=

Variable	No. of patients	%0
Age (year)		
10	14	23.4
11-20	3	5
21-30	9	15
31-40	27	45
41-50	5	8.3
51	2	3.3
Sex		
Male	40	66.7
Female	20	33.3
Residence		
Urban	41	68.3
Rural	19	31.7
Education		36.7
Illiterate	22	
Primary school	21	35
Secondary school	9	15
Collage	8	13.3
Number of lesions		
10	45	75
11-20	6	10
21-30	1	1.7
31-40	1	1.7
41-50	4	6.7
51	3	5
Side of lesion		
Right	45	75
Left	15	25
Location of lesions		
Head and neck	48	80
Genital area	5	8.3
Other sit body	7	11.7

No: Number of patient

4.1.2.Detection of Molluscum contagiosum virus (MCV) and typing

Fifty one 51(85%) of patients skin lesions gave positive result for MCV, and 9 (15%) gave negative result as in (Table 4.2), fig (4-1).

Thirty (58.8%) gave positive result for MCV typing 1 and 2 , 21(41.2%) gave negative result , as in (Table 4.3),fig (4-2).

Table (4.2) Detection of MCV in patients samples by PCR technique.

Result	No. of patients	%
Positive	51	85
Negative	9	15
Total	60	100

Table (4.3) Typing of MCV in patients samples by PCR technique(n=51)

Result	No. of patient	%
Positive	30	58.8
Negative	21	41.2
Total	51	100

n= 51: Only positive result from 60 samples that detection by first PCR..

4.1.3. Distribution of MCV according to following variables:

4.1.3.a. Distribution of MCV according to the age.

The results showed that (23; 45.1%) of patients within age group (31-40 year) positive for MCV , (11; 21.6%) in the age group (10year), (8; 15.7%)in the age group (21-30 year), (4; 7.8%) in the age group (41-50 year), (3; 5.9%) in age group(11-20 year),and(2; 3.9%)in the age group(51 year), (Table 4.4).

Table(4.4.) Distribution of MCV according to the age patients:

Age	No. of patients	%
10	11	21.6
11-20	3	5.9
21-30	8	15.7
31-40	23	45.1
41-50	4	7.8
51	2	3.9
Total	51	100

4.1.3.b. Distribution of MCV according to the sex.

The results showed that MCV was more prevalent in males (36; 70.6%) incompresim with females (15; 29.4%), no significant difference of MCV between both sexes ,(Table 4.5).

Table (4.5.) Distribution of MCV according to the sexes of patients:

Sex	No. of patients	%	P. Value
Male	36	70.6	Ns
Female	15	29.4	
Total	51	100	

Ns: no significant

4.1.3.c.Distribution of patients according to the residence of patients

Most of the patients were from urban area (36; 70.6%) compared with rural group (15; 29.4%) , the difference in residence was statically significant, (Table 4.6).

Table (4 .6) Distribution of pati s according to their residence.

Residence	No. of patients	%	P. Value
Urban	36	70.6	**Sig**
Rural	15	29.4	
Total	51	100	

Sig: significant

4.1.3.d. Distribution of MCV according to the educational level.

The results showed that (18; 35.3%) were illiterate , (17; 33.3%) were of primary school level , (9; 17.6%) were of secondary school level and (7; 13.7) were of college level , no significant difference between MCV according to educational level , (Table 4.7).

Table(4.7) Distribution of MCV according to educational level

Educational level	No. of patients	%	P. Value
Illiterate	18	35.3	Ns
Primary school	17	33.3	
Secondary school	9	17.6	
College	7	13.7	
Total	51	100	

Ns: no significant

4.1.3.e. Distribution of MCV according to number of the lesions.

The study revealed that (40; 78.4%) of patients had lesions (10), (5; 9.8%) had (11-20) lesions, (2; 3.9%)had (41-50) lesions , (2; 3.9%) had (50) lesions , (1; 2%) had (21-30) lesions and (1; 2%) had(31-40) lesions, no significant difference between MCV according to the number of lesion s, (Table 4.8).

Table (4.8) Distribution of MCV lesions according to the number of lesions:

No. of lesions	No. of patients	%	P. Value
10	40	78.4	Ns .
11-20	5	9.8	
21-30	1	2.0	
31-40	1	2.0	
41-50	2	3.9	
51	2	3.9	
Total	51	100	

Ns: no significant

4.1.3.f. Distribution of MCV according to the side of body.

The lesions were predominantly seen on the right side of body (36; 70.6%) in comparism with the left side (15; 29.4%) , which showed significant difference between them ,(Table 4.9).

Table(4.9)Distribution the lesions according to the side of the body of patients:

Side of lesions	No. of patients	%	P. Value
Right	36	70.6	Sig
Left	15	29.4	
Total	51	100	

Sig :significant

4.1.3.g. Distribution of MCV according to the location of the lesions.

The study revealed that (40; 78.4%) of lesions located on the head and neck showed positive results for MCV, then (5; 9.8%) on the genital area and (6; 11.8%) on other body sites, no significant difference between MCV according to the area of lesions , (Table 4.10).

Table (4.10) Distribution of MCV lesions according to their area on the body of patient :

Anatomical area of lesion	No. of patients	%	P. Value
Head and neck	40	78.4	Ns
Genital area	5	9.8	
Other body site	6	11.8	
Total	51	100	

Ns: no significant

4.1.4.Detection of MCV typing and effect of sex and age detected by Bam.H1.

4.1.4.a. Detection type of MCV patients .

MCV type 2 was more prevalent (22; 73.3%) in patients with MCV disease, while MCV type1 represent (8; 26.7%), (Table 4.11).

Table (4.11) The frequency of types of MCV as detected by Bam. HI. restriction enzyme

Type of MCV	No. of patients	%	P. Value
MCV type 1	8	26.7	Ns
MCV type 2	22	73.3	
Total	30	100	

Ns: no significant

4.1.4.b.The frequency of MCV type 1 and 2 according to sex.

The results showed that MCV type 2 was more prevalent in males (14; 46,7%)in camperism to the females patients (8; 26.7%) , while both males and females were affected equally by MCV type1, no significant difference between the types of MCV according to the sex . (Table 4.12).

Table (4.12) Distribution of MCV type 1 and 2 in relation to se› .

Type of MCV	Male		Female		Total	%	P. Value
	No.	%	No.	%			
MCV type 1	4	13.3	4	13.3	8	26.7	Ns
MCV type 2	14	46.7	8	26.7	22	73.3	
Total	18	60	12	40	30	100	

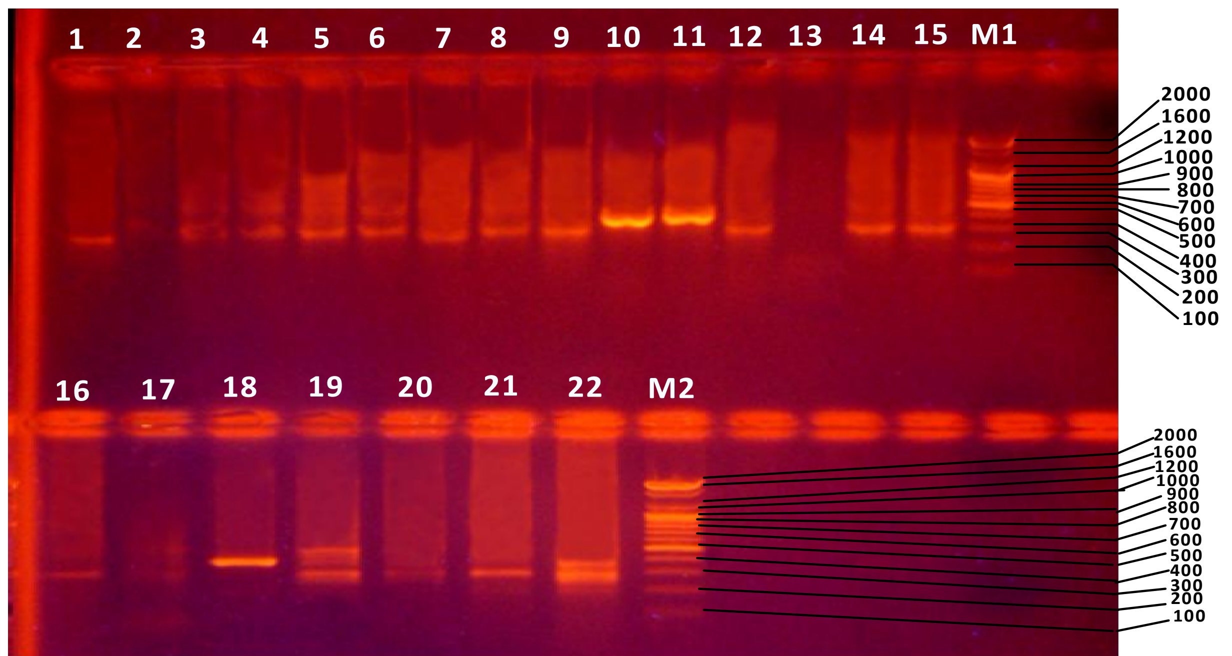
Ns: no significant

4.1.4.c.Distribution of MCV types according to the age of patients.

The results showed that MCV type 1 was more prevalent in the children in the age group (10year) were it consisted of (8; 26.7%) while all cases in this group infected by MCV type1 (100%), and MCV type 2 was more prevalence in adult age group (31-40 year) (14; 46.5%), with significant difference was found between the age and the MCV types . (Table 4.13)

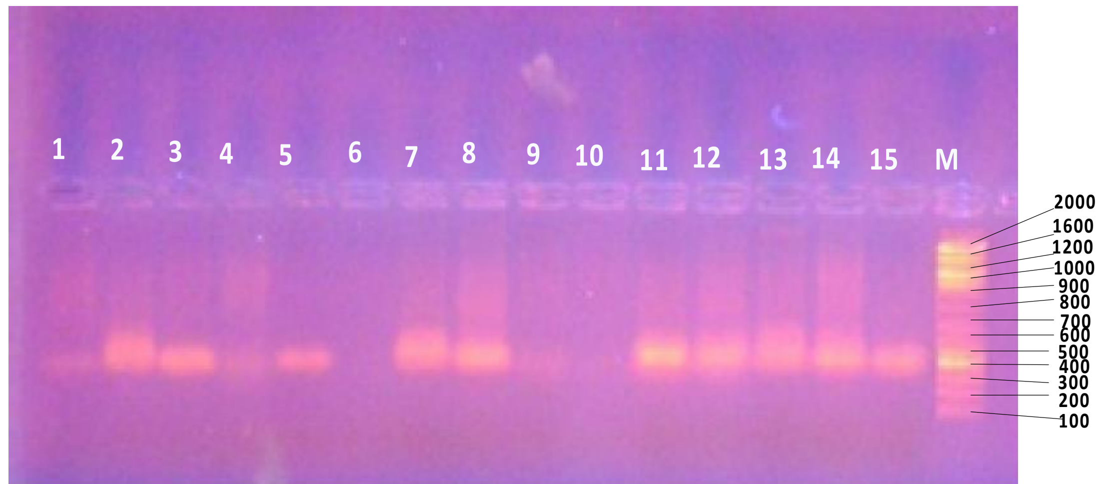
Table(4.13) Distribution of MCV types according to the age of patients .

Age	Type 1		Type 2		
	No.	%	No.	%	Total
10	8	26.7	0	0	8
11-20	0	0	2	6.7	2
21-30	0	0	2	6.7	2
31-40	0	0	14	46.5	14
41-50	0	0	2	6.7	2
51	0	0	2	6.7	2
Total	8	26.7	22	73.3	30



Fig(4-1) Electrophoresis PCR detection of the extracted DNA from (MCV) lesions as (implicated by positive control 393-bp amplicon) for detection of MCV on agarose gel concentration(1.5%),voltage (60)for (90min.)

Lanes 1 = positive result for MCV DNA extracted from sample 25
Lanes 2 = negative result for MCV DNA extracted from sample 36
Lanes 3 = positive result for MCV DNA extracted from sample 10
Lanes 4= positive result for MCV DNA extracted from sample 45
Lanes 5 = positive result for MCV DNA extracted from sample 46
Lanes 6 = positive result for MCV DNA extracted from sample 63
Lanes7 = positive result for MCV DNA extracted from sample 49
Lanes 8 = positive result for MCV DNA extracted from sample 2
Lanes 9 = positive result for MCV DNA extracted from sample 35
Lanes 10 = positive result for MCV DNA extracted from sample 38
Lanes 11 = positive result for MCV DNA extracted from sample 20
Lanes 12 = positive result for MCV DNA extracted from sample 72
Lanes 13 = negative result for MCV DNA extracted from sample 28
Lanes 14 = positive result for MCV DNA extracted from sample 6
Lanes 15 = positive result for MCV DNA extracted from sample 11
Lanes 16 = positive result for MCV DNA extracted from sample 15
Lanes 17 = negative result for MCV DNA extracted from sample 18
Lanes 18 = positive result for MCV DNA extracted from sample 23
Lanes 19 = positive result for MCV DNA extracted from sample 24
Lanes 20 = positive result for MCV DNA extracted from sample 26
Lanes 21 = positive result for MCV DNA extracted from sample 8
Lanes 22 = positive result for MCV DNA extracted from sample 29
M1,M2(100-bp DNA ladder:2000bp, 1600bp,1200bp,1000bp, 900bp,800bp,700bp,600bp, 500bp,400bp ,300bp, 200bp,100bp)



Fig(4-2) Electrophoresis PCR detection of the extracted DNA from (MCV) lesions as (implicated by positive control 595-bp amplicon) for detection of MCV on agarose gel concentration(1.5%),voltage (60)for (90min.)

Lanes 1 = positive result for MCV DNA extracted from sample 25

Lanes 2 = positive result for MCV DNA extracted from sample 35

Lanes 3 = positive result for MCV DNA extracted from sample 10

Lanes 4 = positive result for MCV DNA extracted from sample 45

Lanes 5 = positive result for MCV DNA extracted from sample 46

Lanes 6 = negative result for MCV DNA extracted from sample 63

Lanes7 = positive result for MCV DNA extracted from sample 49

Lanes 8 = positive result for MCV DNA extracted from sample 2

Lanes 9 = negative result for MCV DNA extracted from sample 36

Lanes 10 = negative result for MCV DNA extracted from sample 38

Lanes 11 positive result of implicated DNA sample 20

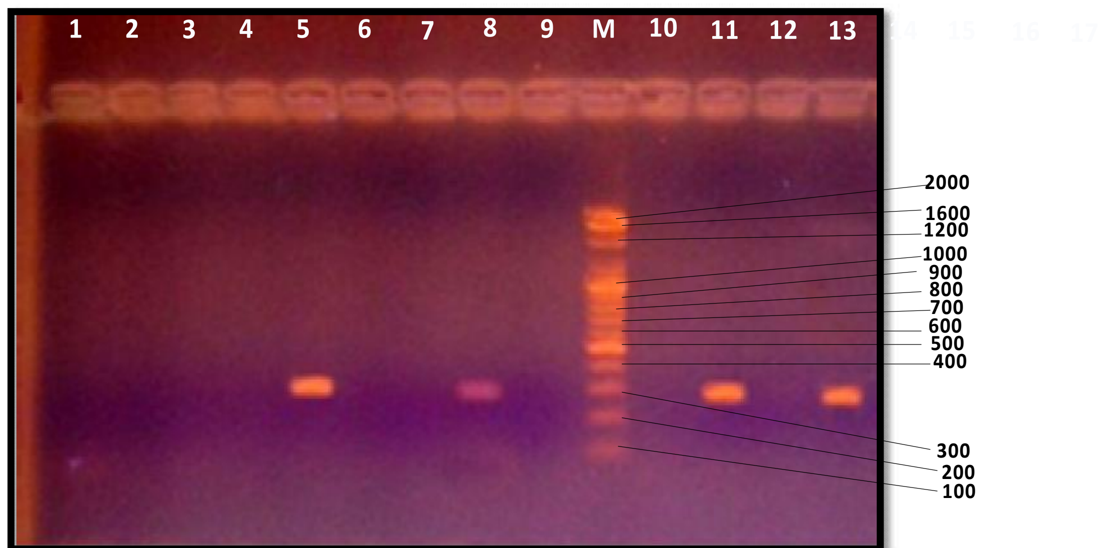
Lanes 12 = positive result for MCV DNA extracted from sample 72

Lanes 13 = positive result for MCV DNA extracted from sample 8

Lanes 14 = positive result for MCV DNA extracted from sample 6

Lanes 15 = positive result for MCV DNA extracted from sample 11

M1 (100-bp DNA ladder: 2000bp, 1600bp,1200bp,1000bp, 900bp,800bp,700bp,600bp, 500bp,400bp ,300bp, 200bp,100bp).



Fig(4-3)BamH1 digestion of positive 575 bp amplicon for subtyping type -1and type -2 on agarose gel concentration(2%),voltage (60)for (90min.)

Lanes 1= type 2(negative result to digestion sample 25 by BamH1)

Lanes 2 = type 2(negative result to digestion sample 36 by BamH1)

Lanes 3= type 2(negative result to digestion sample 10 by BamH1)

Lanes 4= type 2(negative result to digestion sample 36 y BamH1)

Lanes 5 = type 1(positive result to digestion sample **20** by BamH1)

Lanes 6= type 2(negative result to digestion sample 50 by BamH1)

Lanes 7= type 2(negative result to digestion sample 51 by BamH1)

Lanes 8 = type 1(positive result to digestion sample **72** by BamH1)

Lanes 9= type 2(negative result to digestion sample 55 by BamH1)

Lanes 10 = type 2(negative result to digestion sample 70 by BamH1)

Lanes 11 = type 1(positive result to digestion sample **8** by BamH1)

Lanes 12= type 2(negative result to digestion sample 22by BamH1)

Lanes 13= type 1(positive result to digestion sample **6** by BamH1)

Lanes 14 = type 2(negative result to digestion sample 7 by BamH1)

Lanes 15 = type 2(negative result to digestion sample 12 by BamH1)

Lanes 16 = type 2(negative result to digestion sample 21 by BamH1)

Lanes 17= type 2(negative result to digestion sample 27 by BamH1)

M (100-bp DNA ladder:- 2000bp, 1600bp,1200bp,1000bp, 900bp,800bp,700bp,600bp, 500bp,400bp ,300bp, 200bp,100bp).

4.2. Discussion:

4.2.1 Detection of Molluscum cntagiosum virus (MCV) and its typing.

The present study was disagreement with this reported by other researchers (Agromayor *et al .,* 2002 & Yunus *et al.,*2006) , who found that 100% of cases in their study gave positive results.

This difference in result may be due to mutation happened in this region or may be appeared as a new strain, where type 1 and 2 had more than subtype (Porter *et al*, 1992.,Carol *et al.,*1997), three major genomic types with widespread distribution throughout the world had been identified: MCV 1 and its minor variant MCV 1v, MCV 2, and MCV 3, PCR confirm and identify between the two major MCV types (MCV 1 and MCV 2).

This difference in results may be due to a third type that has not been detected in this study or may be due an error occurs in the clinical diagnosis, (Table 4.2, 4.3).

4.2.2.Distribution of MCV according to according to following variables:

4.2.2.a. Distribution of MCV according to age.

This study revealed that most of patients were found in age range (31-40) and that in agreement with the study done by (Chandrshekar *et al.,*2002). Molluscum contagiosum was most commonly seen in the age group (5-10 years), followed by the age group (1-5 years), then age group (10-14years) and less common in age than-1-years, also in agreement with study in USA reported by(Dohil *etal;*2006) in which approximately 80% of the patients was younger than 8 years age, and in disagreement with (Kubchabal *et al.,*2011) ,who reported that (62%-64 %) of patients belonged to ages (11 to 30 years), (Table 4.4) .

This difference in results may be due to the difference in social living levels.

4.2.2.b. Distribution of MCV according to the sex.

The study showed that the percentage of MCV was in agreement with reported by Turkish researcher (Yunus *et al* .,2006) , (40;67.2% in males and 21;32.8% in females) and also in agreement with study reported in Iran researcher (Zandi *et al.,*1999), (29 ;2.45%) in girls and (46 3.1% in boy) and found these differences were not statistically significant, but in disagreement with study in Egypt in which males represented (42.9%) and females (57.1%) ,which was statically no significant (Mostafa *et al*.,2012), (Table 4.5) .

This difference in results may be due our social habits.

4.2.2.C. Distribution of patients according to the residence

The present study was in less agreement with study in USA reported by (Mary *et al* .,2009),who found no statistically significant differences, in terms of region of residence, but the present study was more agreement with (Anna *et al.,*2005& Daniel *et al* .,2003) ,who reported respectively that MCV is more common in hot countries, and among economically deprived communities with overcrowding. MCV affected mainly unskilled workers, and the disease is endemic with a higher incidence within institutions and communities where overcrowding.

This variation in result may be due to, habitats of community condition was living in crowding area, tuvinl and most of patients from Baquba city , (Table 4.6).

4.2.2.d. Distribution of MCV according to the educational level

The results of present study regarding to educational level of patients were in agreement with (Mostafa *et al*.,2012),the incidence among children in public school were more than private school and in children from families with low education and also large families.

The present study was in agreement with study reported by researcher (Kuchabal *et al .,*2011) , that prevalence of MCV was common among student and housewives (74%), followed by unskilled worker (30.7%), agriculturists and businessmen (26.9%),(Table 4.7)

. This difference in results may because the lack of education lead the lack of attention to health, especially when the emergence of such diseases is not just of interest because it is a little knowledge of health matters.

4.2.2.e.Distribution of MCV according to the number of the patients lesions .

The result of present study was more agreement with research reported by (Agromayor *et al* .,2002) no statistically significant differences in number of lesion were detected when comparing individuals with different age, environment, or dermatologic conditions**.**

The present study was in less agreement with study reported by Turkish researchers (Yunas *et al*.,2006*)*,they found that a high number of lesion was seen in age (10years) (24;39.3%) of patients , (Table 4.8).

4.2.2.f. Distribution of MCV according to side of the lesions .

.This result may be related to kissing person to person on right side of face in happy and sad habits ,scratching by right hand of the same side of the face , (Table 4.9).

4.2.2.g. Distribution of MCV according to the location of lesions .

The present study was agreement with study reported by (Bernard., 2001)& (Untoo *et al* .,2009), in adults the MCV lesions were located mainly on the face, whereas in older children they are located on the trunk .The site of predilection of MCV were as follows: head and neck (34.7%), trunk (27.1%), lower limbs (20.7%), upper limbs (8.7%), and genitalia (3.8%) .and in agreement with study reported by (Stulberg., 2003 & Talia et al.,2005), MCV most commonly occurs on the arms, legs, trunk and facial regions .

This study was in disagreement with study done by (Monica etal.,2002), most lesions (64%) seen on the trunk and extremities, whereas face and neck locations were more frequent in adult patients (41%) than in children and young age (16% and 11%, respectively).

This variation in the results was because the exposed sites were more liable to develop the disease , (Table 4.10).

4.2.3 Detection of typing of MCV and effect of sex and age .

4.2.3.a. The frequency of MCV type 1 and 2 detected by Bam. HI restriction enzyme.

The present study was in less agreement with Spanish research reported by (Agromayor *et al* .,2002) ,they found an overwhelming MCV type 1 infection in a population with a ratio146:1 for MCV type1 to type 2 , also in disagreement with Turkish study reported by (Yunas *et al*.,2006) who demonstrated that type1 is the only dominant , and in disagreement with study reported by (Samir .,2009) who found that MCV type in (75%-90% of cases) was the most common, followed by type 2.

This study was in agreement with (Yamashita *et al.,* 1996) his results enhance the authors to suggest a possible difference route of transmission for MCV type 2 involving a sexual contact . (Sara *et al* .,2006) indicated that the widespread of MCV infection may be due to impairment present of immune system.

However, this variation in result was due to increasing number of adults infection with molluscum contagiosum in recent years and the figures were more than doubled during the last 10 yearss , this difference between the incidence of type-1 and type-2 may be as a result of the composition of the community as well as the dominant social relations, , (Table 4.11),fig (4.3).

4.2.3.b.The frequency of MCV type 1 and 2 according to sex .

The result was more agreement with (Scholz *et al.,*2000), where no significant difference was found between type and sex of patients, but in disagreement with that reported by (Jean.,2004) , where the female/male ratio was 1.2:1.

In this study variation in result may be due to that the viral virulence was not affected by the type of sex, there for the disease affected both sex with little variation in rate of infection because most of visitors to outpatient clinic of Baquba Teaching hospital from male . (Table 4.12)

4.2.3.c.Distribution of MCV types according to the age.

This study was more agreement with study reported (Hanson *et al* .,2003),(Magdalene *et al* .,2006)and (Clark.,2009) who found respectively showed that type1 is the common cause of the disease in children, with age group (1-10years), the prevalence of MCV type1 was observed the age, type2 higher in the younger age and adults , approximately 80% of the patients were more than 12 years old, (Table 4.13).

This difference in results also may be due our social habits.

Conclusions and Recommendations

Conclusions

1. Patients with MC infection shows positive results for MCV by PCR.
2. MCV type 1 was high in children with age group (10) , and equal rates in females and males.
3. MCV type 2 was predominately seen in age group(31-40 years) .
4. Most of MCV infections was seen in illiterate level .
5. The right side of head and neck was predominately affected.
6. Infection with MCV in urban area was higher than in rural area .

Recommendations

1. A future study to determine the prevalence of different molecular types of MCV in different sectors of Diyala province .
2. More studies to determine the specific immune response against MCV in patients infected by MCV.

2.Literature review

2.1 Molluscum contagiosum virus :

Molluscum contagiosum virus (MCV) was the one species of poxvirus family, which causes the disease in humans. Virions had a complex structure and was consistent with the structure of the poxvirus family: an envelope, surface membrane, core, and lateral bodies (Büchen *et al.,* 2003).

Most poxviruses were the largest and complex group of viruses, the family included large group of agents that were similar morphologically and shared a common nucleoprotein antigen. Infection with most pox viruses characterized by skin rash, although lesions induced by some members of family are markedly proliferated (Jawetz.,2010).

2.2 Classification of human Molluscum contagiosum:

Pox viruses are divided in to two subfamily based on whether they infected vertebrate or insect host. Pox viruses that infect vertebrates are the subfamily Chordopoxvirinae and share with several biological features ,most of them are large, brick-shaped DNA viruses, with genomes range from 130–375 kilo base pair (kb), and replicated exclusively in the cytoplasm of infected cells (Moss., 2001).

The Chordopoxvirinae was subdivided into eight genera, and members of at least half of these (Orthopoxviruses, Parapoxviruses, Molluskipoxvirus And Yatapox Viruses) can infect human, either exclusively for example, variola virus and MCV (Lewis *et al.,* 2004 & Frey *et al*.,2004). Cobbold and MacDonald saw more patients with venereal infection than paediatric type *Molluscum contagiosum*, and suggested classification of *Molluscum contagiosum* as a sexually transmitted disease. (Ke xing *et al* .,2006)

Table(1.1)Classification of human *Molluscum contagiosum* virus (Jawetz.,2010)

Virus classification	
Group:	Group I (dsDNA)
Family:	Poxviridae
Genus:	Molluscipoxvirus
Species:	*Molluscum contagiosum virus*

2.3.MCV variation and typing.

2.3.1.MCV variation:

Three genomic types of MCV of widespread distribution throughout the world had been identified :MCV-1, MCV-2, and MCV-3. In addition, there was evidence for the existence of at least one further type MCV-4 (Agromayor *et al*.,2002).

The nucleotide composition of genomic DNA was very variable. This variation was not limited to non coding DNA or influencing codon-usage, it also results in changes of the amino acid composition of proteins a Molluscipoxvirus had 35% A+T. MCV-1 had variation in its genome and showed a common difference of genome, this MCV-1 prototype (MCV -1p), reported to be most prevalent in Europe. The common markers of the variants of MCV-1were 24 kb fusion fragments generated by the loss of a Bam. HI site. These variants of MCV-1 were classified into three groups (MCV -1va, MCV -1vb, MCV -1vc), with the variability among them being due to losses of Bam. HI sites located in the right terminus of MCV -1va. Sequencing of PCR products revealed complete homology between MCV-1 and 1v, but minor nucleotide variations between MCV 1/1v and MCV-2 (Jason *et al* 2007) .

MCV has been observed in other species including chickens, sparrows, pigeons, chimpanzees, kangaroos, dogs and horses , was thought to be identical or closely related to human MCV. MCV has never been experimentally transmitted between animals . There was also very limited evidence of transmission between horses and man. The restriction map of MCV-4 was generated and lined up with those of the other types (Thiemann *et al*.,2012).

2.3.2.MCV typing :

There were two main types of MCV, two genetic types MCV-1 and MCV-2, they found in human, all types of MCV had similar clinical presentation and did not localize to a particular region of the body which mean that types and their variants produce lesions that are similar in number, size, and no correlation was found between type and the sex of the patients (Scholz *et al.,*1989& Trama *et al*.,2007).

Restriction endonuclease and PCR analysis of MCV DNA can identified those types MCV-1 and MCV-2. There was considerable conserved genetic organization between MCV-1 and MCV-2. However, there was a12 kilo base

pair region unique to MCV-1 and 2-3 kilo base pair deletion in MCV-2. The G+C content was maintained., MCV-1 was 67% G+C rich in 200 base pair (bp), whereas MCV-2 was 69% G+C rich in 173 bp following the termination codon (Tony *et al*., 2010& sourav *et al*,2011).

Sequences downstream of the MCV genes were also compared, within the first 70 base pair(bp) downstream of the termination codon, there was 75% homology between the sequences of MCV-1 and MCV-2 (Jason *et al*,2007).

2.3.2.a.MCV type one :-

This type was the common cause of the disease incidence in children, with aged one to ten years , MCV -1 was cause the majority of infection (76% to 97%) (Hanson *et al* .,2003& Dixit *et al* 2009).

Children usually acquired Molluscum contagiosum non sexually at both genital and non genital area and the disease was rare under the age of 1 year, perhaps because of transmission of maternal immunity and because of the long incubation period from 2 to 7 weeks with a range extending to 6 months. DNA sequence analysis of the MCV type 1 revealed of genome (185) Kb (Nanhai *et a*l.,2000 ; Silverburg *et al* .,2000 & Simonart *et al*.,2002)

2.3.2.b.MCV type two :

Usually seen in adults, immune deficient (AIDS) and immunosupressed, characterized by benign self-limited eruption of single or multiple cutaneous spherical and pearly papules. Transmission usually occurs by direct contact with infected hosts or used tool like a tattoo tool and also could transmitted sexually. MCV-2 causes the majority (60 %) of infections, in HIV infection-associated molluscum, MCV type 2 genome is about (195)Kb (Odom *et al*.,2000&Molina *et al*.,2011).

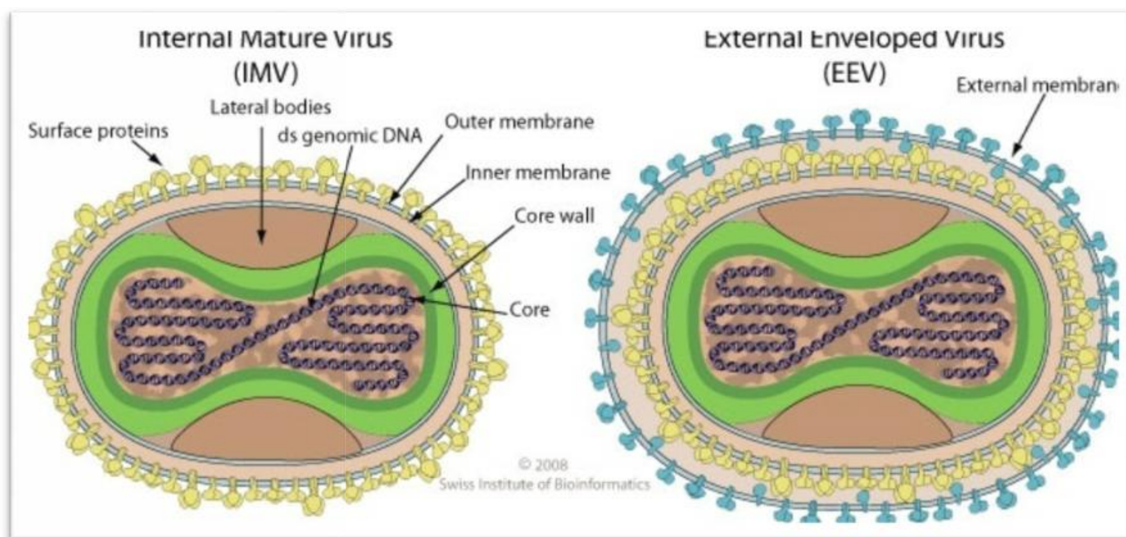
2.4. Molluscum contagiosum structure :-

Mollusciproxvirus has envelope and brick-shaped and is about 320×250× 200 nm (nanometer). Two distinct infectious virus particles exists: intracellular mature virus (IMV) and extracellular enveloped virus (EEV). Linear, dsDNA genome about 190 kb. MCV had collinear genome flanked by inverted terminal repeat (ITR) sequences which are covalently-closed at their extremities (Jawetz.,2010).

MCV was largely with a high molecular weight. The largest among animals viruses, only slightly smaller than the smallest bacteria, and were just visible using light microscopy (Romiti.,2000) (Fig 1.1).

Virions had a complex structure, enveloped, surface membrane, core, the core contains the virus genome and enzymes required for infection and replication. MCV showed similar nucleic acid content and sedimentation behavior to vaccinia virions and lateral bodies. Virus may be contained within inclusion bodies and release by budding through the membrane of the host cell giving rise to a large amount shedding in a short period of time (Büchen.,2003).

The structure of the mature and immature MCV particles inside the envelope become partly rearranged into parallel bands. The mature particles suggest the presence of water-rich regions. The organization of the nucleoid was investigated by the sections analyzed in a tilting rotating specimen stage. The nucleoid consists of fibers with a diameter of approximately 10 nm which are probably connected to each other, thus forming one long fiber. In thin sections four profiles of the nucleoid were observed which were transformed into one another by tilting. Serial sections together with tilting revealed a twist of about 180 degrees between the fibers of the two lobules of the nucleoid. A model for the conformation of the nucleoid in the mature brick stone particles was presented. The inner part of the virion consists of a nucleoprotein core (dumbbell shape in the schematic above) and two proteinaceous lateral bodies. Surrounding these by lipid-containing surface membrane, outside of which are several virus-encoded proteins in structures referred to as ’tubules’, which form a mesh-like coat. This particle was referred to as an intracellular infectious virion. A second form of the virion was found outside the cell and was called the extracellular enveloped virion, which has a second external lipid-containing envelope (Valentine .,2000).



Fig(1.1) The structural shape of MCV(http:/ /www. poxvirus. org., 2012)

2.5 Viral replication :-

Replication of all poxvirus in the cytoplasm of the cell involves of the several stages.The replication of poxvirus is unusual for a virus with double-stranded DNA genome (ds DNA) because it occurs in the cytoplasm (Mutsafi *etal.,*2010).

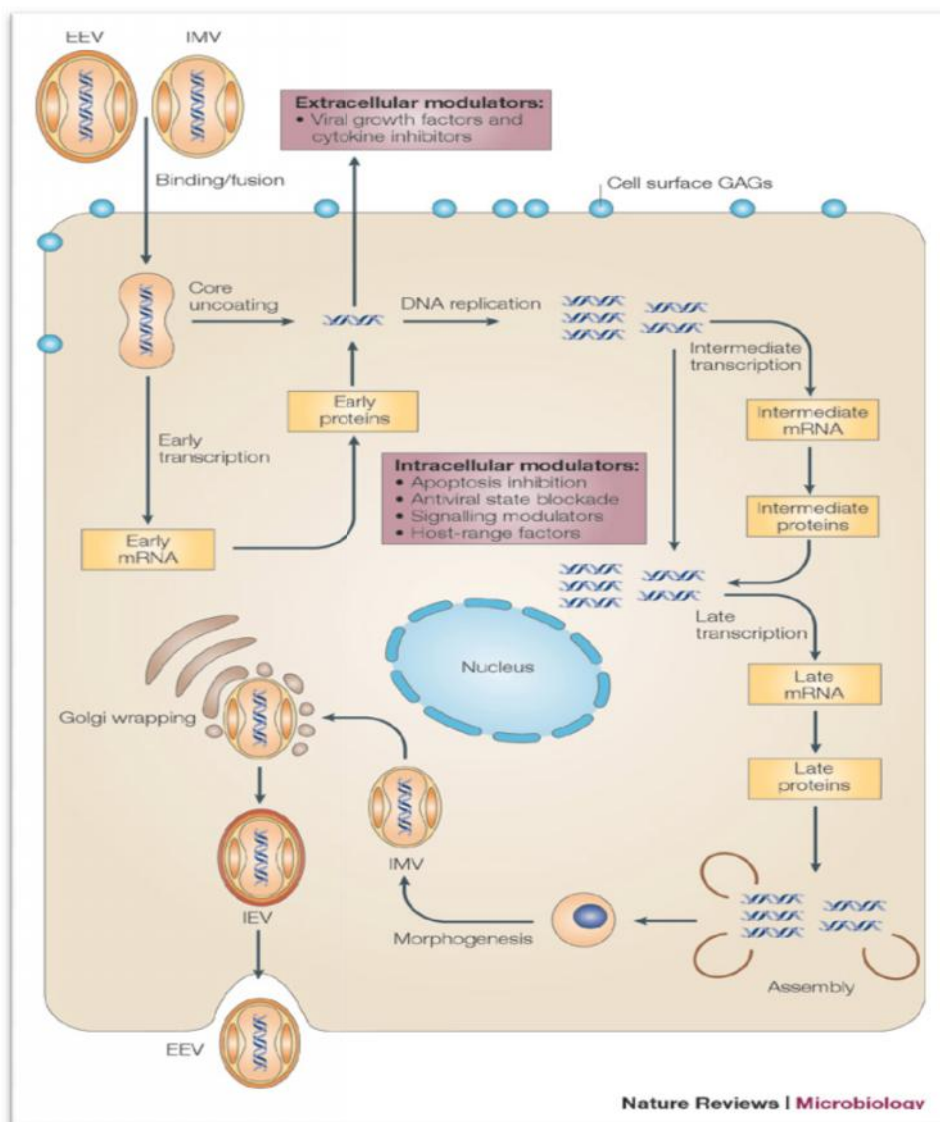
Poxvirus encodes its own machinery for genome transcription, a DNA dependent RNA polymerase, which makes replication in the cytoplasm. Most ds DNA viruses require the host cell's proteins to perform transcription. These host proteins are found in the nucleus, and therefore most ds DNA viruses carry out a part of their infection cycle within the host cell's nucleus. Moreover, genes that had been associated with Vaccinia Pox Virus replication, namely DNA polymerase, uracil DNA glycosylase, topoisomerase, and the putative nucleoside, are highly conserved in MCV . Nevertheless MCV lacks many nonessential genes of other poxviruses that may allow viral replication to occur in resting cells. The missing genes include a DNA ligase, two ribonucleotide reductase subunits, glutaredoxin (O_2L), thymidine kinase ,thymidylate kinase, guanylate kinase, and deoxyuridine triphosphatase. The absence of these enzymes may limit the replication of MCV to metabolically active keratinocytes. In this respect, there is enhanced proliferation of cells in the basal layer of MCV lesions and labeling of viral inclusions with [3H] thymidine appeared most intense in the suprabasal layer and followed a gradient of diminishing labeling through the

stratum spinosum toward the granular layer. MCV had a protein kinase 2, structural proteinVP8, RNA polymerase 35 kilo Dalton subunit and 3beta-hydroxysteroid dehydrogenase (Tatiana *et al*.,1997).

2.5.1.Stages of replication:-

Virus attaches to glycosaminoglycans (GAGs) on the surface of the target cell by components of the extracellular matrix, after binding, the fusion event between the virion and the host cell membranes was still poorly understood, but at least conserved virion protein has been linked to this fusion/entry event that ultimately releases the virion core structure into the cytoplasm (Senkevich,2004) . Although no specific cell receptors are known to be required for virion fusion and entry, there was evidence that virion binding and/or entry was associated with rapid signaling events in several host protein-kinase cascades triggering membrane fusion and release of the virus core into the cytoplasm (Lin *et al.,*2000) (Fig 1.2).

1. Early phase: early genes are transcribed in the cytoplasm by viral RNA polymerase. Early expression begins at 30 minutes post-infection
2. Core is completely uncoated as early expression ends, viral genome is now free in the cytoplasm.
3. Intermediate phase: Intermediate genes are expressed, triggering genomic DNA replication at approximately 100 minutes post-infection.
4. Late phase: Late genes are expressed from 140 min to 48 hours post-infection, producing all structural proteins.
5. Assembly of progeny virions starts probably in association with internal membranes of the infected cell(in the cytoskeleton of the cell), producing an spherical immature particle. This virus particle matures into brick-shaped intracellular mature virion (IMV).
6. IMV can be released upon cell lysis, or can acquire a second double membrane from trans-Golgi and bud as external enveloped virion (EEV).The IMV and EEV virions differ in their surface glycoproteins and in the number of wrapping membranes (Lewis *et al.,*2004) .



Fig(1.2) Poxvirus replication cycle. All poxviruses replicate in the cytoplasm of infected cells by a complex, but largely conserved, morphogenic pathway. Two distinct infectious virus particles - the intracellular mature virus (IMV) and the extracellular enveloped virus EEV(Grant.,2005).

2.6. MCV genome:

The genome is not segmented and contains a single molecule of linear double stranded DNA. The complete genome consisted of 180000-200000 nucleotides long and 160 putative genes had been identified (Thompson., 2000).

The DNA molecule having a mean contour length of 53.02 ± 1.87 µm. The average molecular weight of MCV DNA was calculated to be 118×10^6. When MCV DNA was cleaved by restriction endonuclease Bam.HI,

12 fragments were produced ranging in size from 4×10^5 to 31×10^6 daltons as determined by agarose gel electrophoresis and contour length measurements (Ronald., 2004).

The MCV genome was similar to that of Orthopox viruses (OPV) with regard to size, terminal cross-linking and presence of inverted terminal repeats. Analysis of the MCV genome revealed that it encodes approximately 182 proteins, 105 of which had direct counterparts in Orthopox viruses (OPV) . The MCV genome contains 63.4% guanine + cytosine and hence encodes a low frequency of the stop codons UAA, UAG, and UGA . MCV DNA contains a greater number of long and overlapping open reading frames (ORF) (Yu li *et al* .,2010).

Until recently, only small segments of the MCV genome had been sequenced with the exception of the terminal nucleotides, was available. In initial comparison of the genomes of MCV and OPV indicated that their central regions are conserved , whereas the ends are unique and contain genus-specific host response evasion genes. Lipids are present and located in the envelope. Virions were composed of 4% lipids by weight. The composition of viral lipids and host cell membranes are similar. The lipids are host derived and synthesized denovo (during the early phase of virus replication) and are derived from plasma membranes. Viral membranes include glycolipids (Nakamura *et al*.,1996 & Tatiana .,1997).

2.7.MCV gene products:-

MCV proteins had recognizable cellular homolog's, conserved functional motifs shared with cellular obtains could be detected in several additional proteins, including the poly(A) polymerase catalytic subunit and putative Adenine triphosphatase (ATPases). Some of these proteins, MCV encodes an antioxidant protein MC066L (Molluscum contaiosum 066 Liftward). its homolog's of a glutathione peroxidase, selenoprotein, which functions as a scavenger of reactive oxygen metabolites and protect cells from UV or peroxide damage, homologues to MHC class 1 (Major histocompatibility complex class I) because it's had protein MC080R (Molluscum contagiosum 080 rightward) and also MCV encode two chemokine -like protein MC148R1 (molluscum contagiosum148Rightward) and MC148R2 (Hans *et al*.,2000 & Qingwen *et al.*,2011).

MCV encodes specific molecules to control host defenses by MC159L and MC160L , was predicted to interfere with this cellular response because of its homology to other protein that regulat TNF(tumer necrosise factor) (Danial *et al* .,2006) .

MV159L which as previously showed prevents apoptosis induced by death receptors, also showed to had homology with a family of interlukin-18 binding protein (IL-18BP) with high affinity (Jesus *et al*.,2001 & Yan *et al*.,2003)

MCV often camouflage themselves with host cell membrane by member of poxvirus family mimics CD150 (cluster of differentiation150) receptor. Another MCV encoded protein was MC013L protein inhibits glucocorticoid. Glucocorticoids act as potent inhibitors of keratinocyte proliferation (Nanhai .,2000& Sidorenko *etal*., 2003).

MCV had MC007L protein act with mechanism by which a virus can interfere with the cell cycle. The sequence of MCV has been completed, revealing that MCV encodes a probable type-1 topoisomerase enzyme (Young ,*et al*,1999& Chan *et al*.,2001)**.**

2.8.Envelope Antigen p43k protein :

MCV had a major envelope antigen that called polypeptide 43 kilodalton protein (p43k), it was a late gene transcribed after the onset of DNA replication. This protein was similar to extracellular particles of vaccinia virus bearing envelopes containing the Protein p37K which was an integral part of the envelope of extracellular vaccinia virus and is reported to be associated with the Golgi apparatus within the cell (Hiller *etal.,* 1985 & Hirt, *et al*.,1986).

The (p43k) gene was localized to approximately 30 kb from the left terming of MCV1 and 32kb from the left terming of MCV2 for the isolates used in this study, corresponding to approximately 25 kb from the junction of the left terminal repeat and left unique sequences. The 5' region of the MCV gene for p43K is very G+C rich (72%) overall, but within this region, the A+T-rich sequence TAAAATG encompassed the initiation codon. At position -13 bp from theP initiation codon, there was a string of four thymidine residues. The presence of these two motifs (TAAAATG and TTTT) was more obvious in the G+C-rich MCV genome than in the A+T-rich vaccinia virus genome up stream of the gene for p43K differs from the TAAAT motif

associated with vaccinia virus late genes by the presence of an additional adenine residue (Neil *et al*., 1991).

MCV(p43K) has 42% identity and a further 20% similarity to vaccinia virus p37K at the amino acid level, despite the marked difference in nucleotide composition between the MCV and vaccinia virus DNAs. MCV has a G+C content of 66% (Yuen *et al*.,1984).

2.9. Restriction Enzymes Bam. HI:-

Restriction endonucleases (restriction enzymes) cut DNA molecules at specific position . The designation of these enzymes comes from the source organism and usually these enzymes are isolated from bacteria and are usually named.. Thus, an enzyme from a strain from *Bacillus amyloliquefaciens* is Bam .HI (Jeremy *et al*.,2004 & Anthony *et al*., 2008).

The restriction enzymes, which cut DNA at defined sites, represent one of the most important groups of enzymes for the manipulation of DNA. These enzymes are found in bacterial cells, where they function as part of a protective mechanism called the restriction modification system. In this system the restriction enzyme hydrolyses any exogenous DNA that appears in the cell. Restriction enzymes are presenting three types (I, II, and III). Most of the enzymes commonly used today are type II enzymes, which had the simplest mode of action and the Bam. HI was one of them (Desmond *et al* ., 2008).

Different restriction enzymes generate different ranges of DNA fragment sizes, the size of fragment is linked to the frequency of occurrence of the recognition sequence. Bam HI recognized the sequence 5-GGATCC-3 and the cutting site between the guanine base in the 5- terminal to produce this fragments GATCC-3, and 5-G. These single stranded regions are complementary and will therefore tend to pair with similar regions on other fragments (or at the other end of the same fragment), they are referred to as ‘sticky’ ends (Jeremy *ret al*., 2004) (fig-1.3).

Fig (1.3) Restriction Enzymes Bam. HI(Anthony *et al*.,2008).

2.10.Immune responses to MCV :

MCV was a pox virus that causes tumor like skin lesion occasionally becomes inflamed and regresses spontaneously, an event probably initiated by a host cell-mediated immune rejection against the lesion by plasmacytoid dendritic cells, type 1 interferon production, and dendritic cells had prominent roles in anti-MCV responses and these features characterize the inflammatory response in lesions that will likely undergo spontaneous regression, but it inevitably involves the disruption of the epidermal tissue to expose the molluscum bodies to the tissue fluids of the dermis. It has been suggested that the molluscum bodies induce inflammation by a mechanism similar to that involved in ruptured epidermal cysts or in acne (Melissa *et al*.,2011).

Humoral immunity plays an important role in the body's defense against molluscum infection. Most adults are resistant to MCV infection because they had developed immunoglobulin G (IgG) antibodies against the viral antigen. However, patients with impaired cellular immunity, such as in AIDS or posttransplant immunosuppression, are more likely to develop widespread infections that are difficult to treat (Dohil *et al*,2006& Leena *et al* .,2010).

MCV genome that may interfere with immune recognition and host defense mechanisms include (1) MHC class 1 heavy chain homologue that may inhibit presentation of MCV specific peptides, (2) a chemokine homologue that may inhibit inflammation, and (3) a glutathione peroxidase homologue that may protect the virus and infected cells from oxidative damage by peroxides which may form in response to infection(Moss ., 2000).

Weak immune response to this virus was due to the localization of the antigens in the vascular epidermal tissue, which confers protection from the immune system (Irwin *et al.,*2003).

MCV antibody was found to had a very low level of serum IgM (Immunoglobulin M), elevated levels of IgG and IgA, and a high level of IgE. MCV infected patient had a normal numbers of peripheral blood IgM, IgG, IgA and B-lymphocytes. On the other hand, the patient's T-cells showed normal helper functions and normal interferon (IFN) production in vitro. T-cell functions were impaired, as showed by delayed type cutaneous hypersensitivity (DTH) and mutagen response. The data suggest that the selective IgM deficiency of the patient is due mainly to defects in B-cells at the terminal differentiation stage, but immunological abnormalities are

present in both B-cell and T-cell systems (Mayumi *et al* .,1986& Yang *et al* .,2004).

2.11.Biological, physical and chemical properties of MCV:

Molluscum contagiosum virus propagated in a strain of FL cells (fetal lung cell) of human amnion origin has one step growth cycle time of 12 to 14 h. The appearance and exponential increase of intracellular virus preceded the release of extracellular virus by approximately 2 h. Demonstration of comparable titers of extracellular and intracellular virus at the end of the replication cycle indicated that a substantial amount of virus remained associated with cells exhibiting cytopathogenic changes. Mean buoyant density values of virus in sucrose ranged from 1.275 to 1.278 g/cm^3, but in CsCl the virus banded at densities at 1.325 to 1.340 and 1.261 to 1.281 g/cm^3. Although virus infectivity was not affected by high concentrations of CsCl, it was found by polyacrylamide gel electrophoresis that the salt removed several non glycosylated polypeptides with estimated molecular weights of 15,000 to 60,000 (Pirie *et al.,*1971*).*

The half- life of virus infectivity was approximately 26.5 h at 26Cº and 11.2 h at 37Cº.Although the virus was rapidly inactivated at 50Cº, It could be stabilized at this temperature by present in 1.0 Mgcl$_2$. MCV not agglutinate new borne chicken, adult chicken or type (O) human erythrocyte. Effects by lipid solvent ,PH virus infectivity was found to be sensitive to acid PH-3, but resistant to diethyl ether or chloroform (Robert *et al*.,1976).

The cytopathic activity can be inhibited by pre treatment of virus with specific antiserum trypsin, mechlorethamine hydrochloride, or UV irradiation (La. *et al.,*1976).

2.12.Dermatopathology :-

A key histopathological finding in MCV was the presence of “molluscum bodies” (also called Henderson-Patterson bodies). These are large, round or oval cytoplasmic inclusions containing the molluscum virions as well as an eosinophilic and basophilic structures. These cellular inclusions produce an invasive acanthosis (diffuse hyperplasia and thickening of the epidermis) that causes the surface of the epidermis to slough and form a

central cavity, resulting the characteristic molluscum lesion) (Joseph *et al.,*2011)

The molluscum bodies in the basal layer represented an enlarged basophilic nuclei and mitotic figures are seen. Progressing upward, the cells showed cytoplasmic vacuolization and then eosinophilic globules. The nucleus becomes compressed at the level of the granular cell layer, and the molluscum bodies los their internal structural markings. Undisrupted lesions showed an absence of inflammation, but dermal changes can include an infiltrate of lymphohistiocytic, neutrophilic, or granulomatous and has been seen latter in solitary lesions (Billstien *et al.,*1990).

2.13. Pathogenesis :-

This disease was transmitted primarily through direct skin contact with an infected individual. Fomites had been suggested as another source of infection, with Molluscum contagiosum reportedly acquired from bath towels, tattoo instruments, and in beauty parlors and Turkish baths. The average incubation time is between 2 to 7 weeks with a range extending to 6 months. The virus seems first to enter the basal epidermis where an early increase in cell division extends into the suprabasal layer (Simonart *et al* .,2002) .

The cellular proliferation produces lobulated epidermal growths which compress the papillae until they appear as fibrous septa between the lobules, which are pear shaped with the apex upwards. The basal layer remains intact. These molluscum bodies contain large numbers of maturing virions. These were contained intracellularly a collagen lipid rich sac like structure that was thought to determine immunological recognition by the host. Rupture and discharge of the infectious virus packed cells occur in the center of the lesion. MCV induces a benign tumor instead of the usual necrotic pox lesion infection with the virus causes hyperplasia and hypertrophy of the epidermis. Free virus cores had been found in all layers of the epidermis. So-called viral factories were located in the malpighian and granular cell layers (Tony *et al* ,2010).

2.14. Epidemiology :

MCV has a worldwide incidence between 2-8%. It accounts for approximately 1% of all dermatologic diagnosis (Mayur *et al.,*2008 & Lydia *et al*., 2011).

In study in Iraq they found that the prevalence of MCV is 1.9% of the viral diseases (Khalf *et al* .,2007).

MCV infection with a higher distribution in the tropical areas. A high incidence of MCV infection was documented in a report that 39% of individuals over 50 years of age representative. A history of eczema was found in 62% of children with Molluscum contagiosum in Australia (Braue .,2005).

Prevalence was 2-10% of children annually in the world (Husar., 2002).The incidence of Molluscum contagiosum infections in young children between 2-12 years of age is approximately 17%. Most of these incidents had been reported from hot and humid tropical countries. Child between the ages of 2 to 12 are more prone to the disease. When a child had Molluscum contagiosum, small bumps appear on the legs, body, arms and even the eyelids. These bumps were dome shaped and look like pearl (Clark ., 2009 &Magdalene *et al* 2006).

However ,to a greater extent of Molluscum contagiosum was seen quite commonly on the genital, perineal and surrounding skin of children, and abuse should not be regarded as likely unless there were other suspicious features. There was a clinical impression that Molluscum contagiosum was commoner in patients with atopic eczema, and occasional reports describe widespread infections, possibly based on impaired immunity topical steroids and also topical calcineurin inhibitors had been suspected as a contributing factor in eczema and other patients. The disease is endemic with a higher incidence within institutions and communities where overcrowding, poor hygiene, and poverty potentiate its spread. Over the last 30 years its incidence had been increasing, mainly as a sexually transmitted disease, and it was particularly rampant as a result of concurrent human immunodeficiency virus (HIV) infection (Fery *et al* .,2007).

In a Japanese study involving over 7,000 children, the incidence MCV was 5-7% among swimmers and 3-6% among non-swimmers. Outbreaks may occur among children who bathe or swim together in the United States, from 1990 to 1999 the estimated number of physician visits for Molluscum contagiosum was 280,000 per year (Molino., 2004).

2.15.Transmission of MCV:-

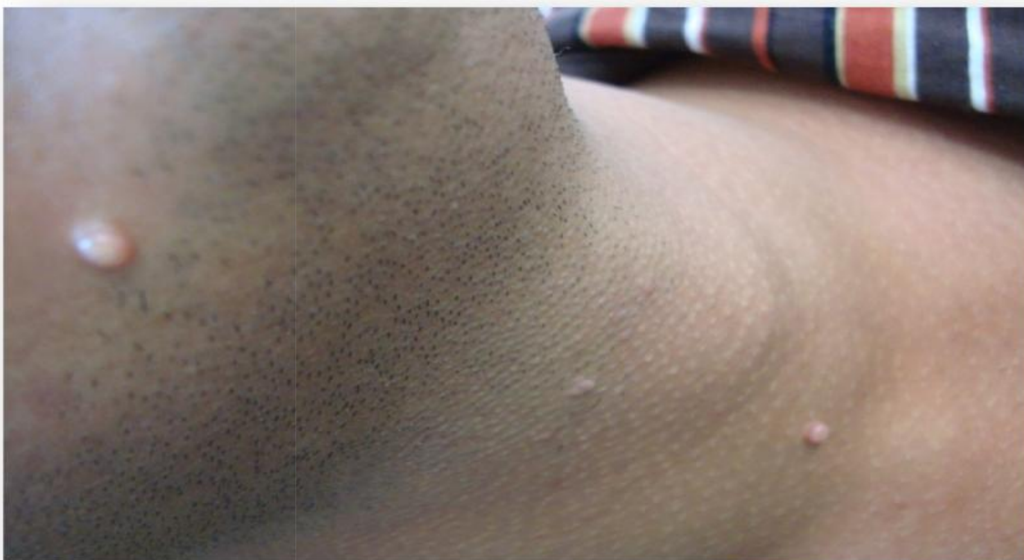
Transmission occurs by person-to-person skin-to-skin contact sports (especially wrestling), or simply touching or via fomites ,towels, bedclothes, clothing including underwear, soft toys, shaving utensils, electrolysis

equipment, tattooing tools, and sponges. The virus may be spread to other areas via self inoculation ,scratching, shaving, or touching a lesion, also in the adult transmition through sexual contact (Stulberg *et al*.,2003& Pickering *et al*., 2009).

Molluscum contagiosum virus (MCV) infection induces self limiting cutaneous lesions in an immunocompetent host that can undergo spontaneous regression preceded by local inflammation(Janiene *et al.*,2010& William *et al* .,2011)

2.16.Clinical feature :

The incubation period is variously estimated at 14 days to 6 months. The individual lesion is a shiny, pearly white, hemispherical, umbilicated papule which may show a central pore. It may be identified with a hand lens or dermatoscope when less than 1 mm in diameter. Enlarging slowly it may reach a diameter of 5–10 millimeter in 6–12 weeks (Joseph *et al.,* 2010).



Fig(1.4) Clinical feature of *Molluscum contagisum* virus.(personally obtained by courtesy of Dr .Alayally, Kh.Kh ,2012)

Rarely, and usually when one or very few are present, a lesion may become considerably larger. Plaques composed of many small lesions (‘agminate form or giant molluscum) occur rarely. Lesions frequently spread and the number of lesions ultimately present sometimes very large. After trauma, or spontaneously after several months, inflammatory changes result in

suppuration, crusting and eventual destruction of the lesion (Zulfugar *et al.,*2009) .

The duration both of the individual lesion and of the attack is very variable and although most cases are self limiting within 6–9 months, it is not unusual for some to persist for 3 or 4 years ,individual lesions are unlikely to persist for more than 2 months. Patients with HIV/AIDS and other immunocompromising conditions e.g., solid organ transplant recipients can develop “giant” lesions (15 mm in diameter), larger numbers of lesions, and lesions that are more resistant to standard therapy. The following diseases should be considered in the differential diagnosis of Molluscum contagiosum: cryptococcosis, basal cell carcinoma, keratoacanthoma, histoplasmosis, coccidioidomycosis, and *verruca vulgaris* (Hunter *et al .,*2002 & Mansur *et al.,*2004).

2.16.1-Complications:

Complications of MC include irritation, inflammation, and secondary infections. Lesions on eyelids may be associated with follicular or papillary conjunctivitis, bacterial super infection may occur but was seldom of clinical significance .

1. Dermatitis for example (eczema) patches of infective eczema occasionally developed around lesions of Molluscum contagiosum provide a good example, because the pearly papules were the initiating event, and eczema can develop in the surrounding skin some days later, even when the lesions had not been scratched or traumatized. The eczema generally clears when the molluscum lesions subside and it’s called eczema molluscatum (Luciana *et al*.,2009)
2. Hyper-IgE syndrome (hyper immunoglobulin E), the disease had a broader range of bacterial infections along with severe chronic Molluscum contagiosum and herpes infections The key laboratory finding is the great elevation of serum IgE levels (greater than 200 IU/mL), often with some eosinophilia (Lei *et al* .,2006).
3. Cellulitis was an unusual complication of Molluscum contagiosum in patients who are HIV infected.⁻Secondary infection with *Staphylococcus aureus* has resulted in abscess formation, whereas *Pseudomonas aeruginosa* can cause necrotizing cellulitis (Freeman *et al*., 1995& William *et al .,* 2011).

4. In the immunocompromised e.g. in HIV infection, lesions may become large and exuberant, and secondary infection may be problematic (United Kingdom National Guideline ., 2007) .
5. Conjunctivitis and keratitis (Murray.,2004 & Tissa *et al*.,2005).

2.17.Diagnosis of MCV:-

The clinical appearance of Molluscum contagiosum was in most cases diagnostic. Though molluscum cannot be cultured in the laboratory, histological examination of a curetted or biopsied lesion can also aid in the diagnosis in cases that are not clinically obvious. The thick white central core can be expressed and smeared on a slide and left unstained or stained with Geimsa or Gram and Wright, to demonstrate the large brick-shaped inclusion bodies (Bagher *et al .,*2007).

Electron microscopy has also been used to demonstrate the poxivirus structures. Immunohistochemical methods using a polyclonal antibody allows recognition of Molluscum contagiosum in fixed tissue. In situ hybridization for MCV DNA has also been utilized MCV (Daniel *et al .,*2003).

Another methods for detecting MCV and differentiating types from DNA extracts of swab specimens. Previous molecular methods for identifying and genotyping MCV utilized laborious DNA hybridization and restriction mapping techniques. Real-time PCR and Pyrosequencing provide a high throughput means of detecting MCV DNA and acquiring DNA sequence (Jason *et al.,*2007).

2.17.1.Electron microscopic diagnosis of MCV:

Electron microscopy allows rapid morphologic identification and differential diagnosis of different agents contained in the specimen. Details for efficient sample collection, preparation, and particle enrichment are given. Applications of diagnostic electron microscopy in clinically or epidemiologically critical situations as well as in bioterrorist events are discussed. Electron microscopy can be applied to many body samples and can also hasten routine cell culture diagnosis (Paul *et al* .,2003).

On electron microscopy numerous intracytoplasmic viral particles were demonstrated, thus confirming the cytological diagnosis. Clinically unsuspected cases, the cytological diagnosis of Molluscum contagiosum can be suggested by demonstrating pathognomonic molluscum bodies in aspirated material. A specimen can be ready for examination and an

experienced virologist or technologist can identify, by electron microscopy, a viral pathogen morphologically within10 minute. Microscopic examination revealed hypertrophied keratinocytes with cytoplasms occupied by a large intracytoplasmic granular mass. *Molluscum contagiosum* virus detected by electron microscope. EM is the method used for laboratory diagnosis (Constance *et al.,* 2006).

2.17.2. In situ hybridization (ISH):-

In situ hybridization is usually applied to histological sections or cell smears. If performed carefully it can provide not only the exact localization of target sequences, but also excellent morphologic details of tissues or cellular contents (Unger, 2000).

Basically tissue sections or cells permeabilized by a gentle process involving enzymes, detergents, or both so that small pores permit access of probes to nucleus. Hybridization of probe to target occurs within the permeabilized nucleic or other organelles. Although morphology is retained generally intact, some distortions do occur, and these depend for most part on the method of tissues fixation and the nature of the permeabilization process. Various DNA hybridization techniques had been used to identify the presence of MCV in clinical specimens (Nagai *et al.,* 2000 & Jain *et al.,* 2001).

2.17.3.Utility of a Squash Preparation:

Diagnosis can be obtained with biopsy or cytology. The presence of uniform, acidophilic, Henderson-Paterson bodies is pathognomonic for MCV (Frenkl *et al* .,2007).

Biopsy could be performed to determine the diagnosis, but this technique is more costly, invasive, and takes more time than in office squash preparation. In this case we described the technique and utility of squash preparation using Giemsa stain for in office diagnosis of mulloscum contagiosum. In addition to Giemsa, other staining techniques such as Wright, 10% potassium hydroxide and Gram had been described in literature (Bauer *et a*l., 2007).

2.17.4. Real-Time PCR Assays(Quantitative assay):-

Real-time PCR and Pyrosequencing provide a high-throughput means of detecting MCV DNA and acquiring DNA sequence, which is most precised method for discriminating among variants. Real-time PCR assays can be used

for the rapid, sensitive, and specific detection of MCV and it was quantitative assay also known as q PCR, real-time detection of PCR products was made possible by including in the reaction a fluorescent molecule that reports an increase in the amount of DNA with a proportional increase in fluorescent signal. The fluorescent chemistries employed for this purpose include DNA-binding dyes and fluorescently labeled sequence specific primers or probes (Michael *et al*.,2001 & Ahmadian;2006).

Pyrosequencing is a DNA sequencing technology based on the real-time monitoring of DNA synthesis by bio luminescence (Ahmadian *et al*., 2006).

When combined with Pyrosequencing, can further discriminate between MCV1 and MCV2.The use of real-time PCR coupled with Pyrosequencing eliminates these errors by providing exact nucleotide sequences within 30 min post amplification. They were used two dual-labeled probe, one homologous to the p43K gene and one to the MC080R gene, were designed. The p43K PCR was designed to be used in conjunction with Pyrosequencing for confirmation of PCR products and discrimination between MCV1 and MCV2. Both PCR assays were optimized with respect to reaction components, thermocycling parameters, and primer and probe concentrations. The specificities of both PCR assays were confirmed by non amplification of human pathogens, testing 703 swabs, concordance between the two real-time PCR assays was 99.9%. Under the developed conditions, Pyrosequencing of the p43K PCR product was capable of providing enough nucleotide sequence to definitively differentiate MCV1 and MCV2 (Jason *et al*.,2007).

2.17.5.PCR amplification assay(Conventional method):

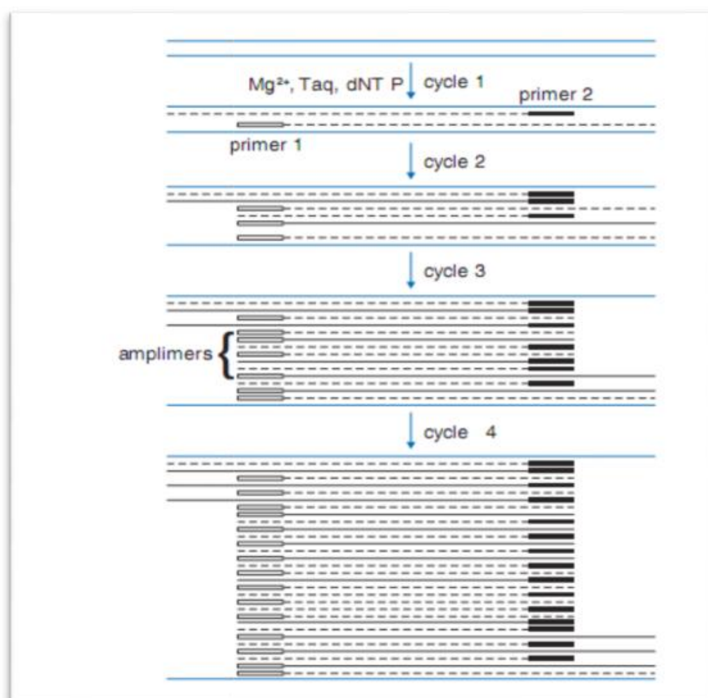
The PCR principles, which were first described in 1986 by Kary Mullis, had revolutionized the way molecular biology is being carried out. In 1993 Noble prize for chemistry was awarded to Dr. Mullis who invented PCR (Bartlett *et al* .,2003).

PCR amplification was in fact a cyclical process where the sample DNA was initially denatured in order to unwind and separate the DNA double helix strands into single strands, usually achieved by heating DNA sample in an aqueous environment, at temperature of 94°C for 30 seconds to 5 minutes (Pavlov *et al.,*2004) .

Hybridization of the specific oligonucleotide primers to each strand was then achieved by lowering the temperature of the reaction mix to the

annealing temperature usually set between 40°C and 65°C (dependent on the design of the oligonucleotide sequences used as primers). After the primer hybridization step, the temperature is raised to approximately 72°C, (an optimal temperature for thermos table DNA polymerase mediated DNA strand replication), and the whole cycle is then repeated a predetermined number of times. After each cycle of replication, each newly synthesized double stranded DNA molecule (known as an amplimer or amplicon) contains terminal sequences, which are complementary to the primer sequences used (Fig1.5). This process allows each amplimer to serve as a template for replication in subsequent rounds of PCR cycling, resulting in a theoretical doubling(exponential amplification)of the number of target molecules during each cycle (Elizabeth., 2008).

Polymerase chain reaction (PCR) technique, demonstrated to be capable of detecting very low amounts of viral DNA in either fresh and formalin-fixed specimens, is described. The primers used were designed to amplify (Faoud *et a*l.,2008) .



Fig(1.5) Schematic representation indicating the principle of PCR and its key components. (Elizabeth *et al* .,2008).The duplication of a region within a DNA target molecule is facilitated by the specific hybridisation of two different oligonucleotide primers (primer 1 and primer 2). A thermostable DNA-dependent DNA polymerase recognises these primers and extends the DNA strand in the 5- to 3- direction while consuming dNTPs. Repeated cycles of strand separation by heat denaturation (melting), primer hybridisation (annealing) and new DNA strand synthesis (elongation) results in the expo-nential amplification of a specific DNA region, as defined by the user designed primers. In general the minimum number of cycles of PCR performed is 20 cycles with 50 cycles usually being regarded as an upper limit (Elizabeth *et al* .,2008).

2.17.5.a.Applications of PCR:

PCR is extensively used in analysing clinical specimens for the presence of infectious agents, like viruses ,bacteria. PCR was particularly high specificity to the early detection of HIV as it can identify the DNA of the virus within human cells immediately following infection, as opposed to the antibodies that are produced after weeks or months of infection (Yang *et al* .,2004& Usman *et al* .,2009)

PCR can also be used to determine the viral load, which was a useful measure of prognosis. It replaces conventional methods of using a specimen to grow the bacteria in the laboratory, which take at least 24 hours. PCR provides a rapid, sensitive and specific alternative. The paternity test was essentially carried out by PCR. A cheek swab was taken from inside the mouth of both parents and the child. The DNA was extracted from the cells obtained and was analyzed by PCR and it used in the forensic science (Mohini *et al.,*2010).

PCR was valuable tool it can provide information on a patient's prognosis, and predict response or resistance to therapy. Many cancers are characterized by small mutations in certain genes (Abu-Duhier *et al*. 2000).

Important application of PCR for the analysis of mutations that occur in many genetic diseases. PCR fingerprinting was a valuable tool for medical microbiologists, epidemiologists, and microbial taxonomists. The current state of PCR-mediated genotyping is reviewed, and a comparison with conventional molecular typing methods is included. Because of its speed and versatility, PCR fingerprinting will play an important role in microbial genetics, epidemiology, and systematic (Bartlett *et al*,2003).

2.18. Prevention and treatment of MCV:-

To prevent MCV transmission avoid direct contact with the skin lesions avoiding sex action also prevent molluscum virus and other sexual transmitted diseases to limit the transmission of disease for children, parents should be advised to avoid taking their children to public bathing or swimming pools, especially during known outbreaks of attendees. Also, children with Molluscum contagiosum should be bathed separately from their siblings and should not share towels or bath sponges with other individuals. In fact, any towel of an infected child uses should be put immediately into the laundry to

prevent infection of the child or spread of disease to another family members (Cohen *et al* .,2004).

Also must Keeping the hands clean can help prevent spreading the virus, covering the bumps by a bandage to cover your bumps if there's a possibility that another person may contact with infected skin. It's not clear if the Molluscum contagiosum virus can spread in the chlorinated water found in swimming pools (Pickering,2009).

To treat MCV many theraptic methods had been used in treatment of MCV and no single treatment or particular combination of therapies is appropriate in all cases. The choice should be based on a variety of factors and tailored to individual cases. As noted above, spontaneous involution of MCV lesions occurs within 2 to 4 years that is, all, or almost all, lesions will clear within 4 years, although many clear much earlier (Jean *etal.,*2004 & Zabawski *et al* .,2000)

Imiquimod 5% For treating large numbers of small lesions. Studies had indicated that it was successful in about 80% of the time. Pulsed dye laser has been reported as being very effective in clearing MC lesions (Hengge *et al*.,2000 &Hancox JG. *et al* .,2003).

Studies showed 96%–99% of the lesions resolved with one treatment Curettage diathermy and Cryotherapy apply liquid nitrogen until a halo of ice.(Hammes.,2001& Sonnex *et al*.,2011).

Many topical preparation which include tretinoin, cantharidin and salicylic lactic acid wart paints and shaving of lesion. Essential Oils was used to treatment MCV in children. In 2004 demonstrated a reduction in the number of lesions in 9 out of 16 children treated once daily for 30 days with essential oil of Australian lemon myrtle (Backhousia citriodora) dissolved in olive oil (Burke *et al* .,2004 & Brown *et al* .,2006).

More recent study showed a 90% or greater reduction in the number of visible molluscum lesions in 16 of 19 children (84.2%) using a combination of essential oil of Melaleuca alternifolia (tea tree oil) and organically bound iodine. Tea tree oil alone worked for only 18% of children (Markum *et al*.,2012).

Iraqi study in 2007 Khalf., described a new simple ,relatively painless and cheep method for treatment ofh MCV by puncturing of lesion by disposable syringe needle (Khalf *et al*., 2007).

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Summary

The present study was conducted for the period from1 November 2011 to April 30of 2012 in outpatient clinic of Baquba Teaching Hospital in Baquba city .The study aimed to confirm the clinical diagnosis of MCV by laboratory test through using PCR assay and to know the domain subtype of MCV that found in Diyala Governorate.

Sixty (60) patients were diagnosed with clinical lesions of MCV on different areas of the body, age of patients ranged from(1-80 years) including 40(66.7%) males and 20(33.3%) females.

After the examination by PCR, 51(85%) of patients gave positive results to MCV and 30(58.8%) patients gave positive results to detected MCV type 1 and 2 .

The results showed 23(45.1%) with age group (31-40 years), included 36(70.6%) were male and 15(29.4%) female, no stasticl significant difference showed between the MCV infection and either the sex or age .

The results revealed MCV type 2 was more prevalent 22(73%) compared with MCV type 1 ,most of type 2 infected males (14;46.5%) , and found in age group (31-40 years), while the MCV type 1 was equally affecting males and females , consisted of (100%) in age (10 years) ,with stasticl significant difference recorded between the types and age, but no significant difference between the types and the sex.

This study revealed that 18(35.3%) were illiterate without stasticl significant difference between MCV infection and educational levels, 40(78.4%) of lesions located on the head and neck, 36(70.6%) on the right side of body, and 40 (78.4%) patients had lesions ranged (10) lesions. There was no stasticl significant difference between infection and area and number of MCV lesions. Location of lesions showed statically significant difference with viral infection , 36(70.6%) lived in the urban, and showed statically significant difference to infection by MCV as compared to rural infected patients.

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List of Abbreviations

Bam.H1	*Bacillus amyloliquefaciens*
bp	base pair
CD150	Cluster of differentiation150
CsCl	Caesium chloride
DTH	Delayed type hypersensitivity
EEV	Extracellular enveloped virus
FL cells	Fetal lung cell
IFN	Normal Interferon
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-18BP	interlukin-18 binding protein
IMV	intracellular mature virus
kb	Kilo base pair
MCV	*Molluscum contagiosum* virus
MC007L	Molluscum contagiosum 7Leftward
MC013L	Molluscum contagiosum13Leftward
MC066L	Molluscum contagiosum 066Leftward
MC148R1	Molluscum contagiosum 148Rightward
MCV -1va	Molluscum contagiosum type 1 versus a
MCV -1vb	Molluscum contagiosum type 1 versus b
MCV -1vc	Molluscum contagiosum type 1 versus c
MCV1	Molluscum contagiosum virus type 1
MCV2	Molluscum contagiosum virus type 2
MCV3	Molluscum contagiosum virus type 3
MCV4	Molluscum contagiosum virus type 4
MHC class 1	Major histocompatibility complex class I
nm	Nanometer
p37K	37 kilodalton protein

p43k	43 kilodalton protein
PBS	Phosphate buffer solution
PCR	Polymerase chain reaction
pmol	picomol
Real- time PCR	Real –time polymerase chain reaction
RPS	Round per second
TNF	Tumor necrosis factor
µm	Micrometer